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removal, customized scheduling. See HELP ALERT.

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            5194  RIBOZYME
           13889  CASSETTE
      S1      75  RIBOZYME AND CASSETTE
? s s1 and py<1999
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        21931987  PY<1999
      S2      37  S1 AND PY<1999
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      S3      22  RD (unique items)
? t s3/3,ab/all

 3/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10158393   99147527   PMID: 10023446
  High level inhibition of HIV replication with combination RNA decoys
expressed from an HIV-Tat inducible vector.
  Fraisier C; Irvine A; Wrighton C; Craig R; Dzierzak E
  Erasmus University Rotterdam, Department of Cell Biology, The
Netherlands.
  Gene therapy (ENGLAND) Dec 1998, 5 (12) p1665-76, ISSN
0969-7128  Journal Code: 9421525
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  Intracellular immunization, an antiviral gene therapy approach based on
the introduction of DNA into cells to stably express molecules for the
inhibition of viral gene expression and replication, has been suggested for
inhibition of HIV infection. Since the Tat and Rev proteins play a critical
role in HIV regulation, RNA decoys and ribozymes of these sequences have
potential as therapeutic molecular inhibitors. In the present study, we
have generated several anti-HIV molecules; a tat-ribozyme, RRE, RWZ6
and TAR decoys and combinations of decoys, and tested them for inhibition
of HIV-1 replication in vitro. We used T cell specific CD2 gene elements
and regulatory the HIV inducible promoter to direct high level expression
and a 3' UTR sequence for mRNA stabilization. We show that HIV replication
was most strongly inhibited with the combination TAR + RRE decoy when
compared with the single decoys or the tat-ribozyme. We also show
that the Tat-inducible HIV promoter directs a higher level of steady-state
transcription of decoys and inhibitors and that higher levels of expression
directly relate to increased levels of inhibition of HIV infection.
Furthermore, a stabilization of the 3' end of TAR + RRE inhibitor
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ribozyme. Second, cultured T lymphocytes expressing an anti-HIV **ribozyme** were challenged with HIV. In both cases, we found that the ribozymes were effective only when expressed as capped, polyadenylated RNAs transcribed from Pol II cassettes that generate a cytoplasmically localized **ribozyme** that facilitates co-localization with its target. We also show that the inability of the other cassettes to support **ribozyme** -mediated inhibitory activity against their cytoplasmic target is very likely due to the resulting nuclear localization of these ribozymes. These studies demonstrate that the **ribozyme** expression **cassette** determines its intracellular localization and, hence, its corresponding functional activity.

3/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08881540 96239508 PMID: 8657127
Role of polyadenylation in nucleocytoplasmic transport of mRNA.
Huang Y; Carmichael G C
Department of Microbiology, University of Connecticut Health Center, Farmington, 06030, USA.
Molecular and cellular biology (UNITED STATES) Apr **1996**, 16 (4) p1534-42, ISSN 0270-7306 Journal Code: 8109087
Contract/Grant No.: CA45382; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
To examine the role of polyadenylation in the nuclear export of mRNA, we have replaced the poly(A) signal in a Rev-responsive human immunodeficiency virus type 1-based reporter gene with a cis-acting hammerhead **ribozyme** . Transcripts from this gene thus acquire a 3' terminus by cis-**ribozyme** cleavage rather than by polyadenylation. The nuclear and cytoplasmic distribution of transcripts was investigated using transient gene expression and quantitative RNase protection assays. In the absence of Rev, a basal level of polyadenylated unspliced mRNA transcribed from a poly(A) signal-containing control reporter gene was detected in the cytoplasm of transfected COS7 cells. However, cytoplasmic **ribozyme** -cleaved unspliced RNA was only barely detectable. The nuclear/cytoplasmic (n/c) ratio of polyadenylated RNAs was 3.8, while the n/c ratio for **ribozyme** cis-cleaved RNAs was 33. The cytoplasmic localization of the polyadenylated unspliced mRNA was enhanced about 10-fold in the presence of Rev and the Rev-responsive element. In marked contrast to this, **ribozyme** cleaved RNA accumulated almost exclusively (n/c ratio of 28) in the nucleus in the presence of Rev. Actinomycin D time course analysis suggested that the low levels of the cytoplasmic **ribozyme**-cleaved RNAs in both the presence and absence of Rev were due to serve export deficiency of **ribozyme** -cleaved RNA. Finally, by inserting a 90-nucleotide poly(A) stretch directly upstream of the **ribozyme** **cassette** , we have demonstrated that a long stretch of poly(A) near the 3' end of a **ribozyme**-cleaved transcript is not sufficient for directing mRNA export. Taken together, these results suggest that polyadenylation is required for the nucleocytoplasmic transport of mRNA and that Rev interaction with the Rev-responsive element cannot bypass this requirement.

3/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08851356 96186746 PMID: 8627795
Adenovirus-mediated expression of ribozymes in mice.

Department of Neuropharmacology, Scripps Research Institute, La Jolla, CA 92037, USA.
Antisense research and development (UNITED STATES) Fall **1995**, 5 (3) p203-12, ISSN 1050-5261 Journal Code: 9110698
Contract/Grant No.: AI-32935; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Ribozymes catalytically cleave substrate RNA molecules in a sequence-specific manner. Engineered ribozymes can be developed and introduced into tissue culture cells to regulate gene expression and to inhibit viral replication. We have previously reported on the construction of cell lines that constitutively express a single antiviral **ribozyme** embedded in a lengthy RNA transcript. These cells exhibited a marked reduction in their ability to support viral infection. Here we report the construction of RNA molecules that contain one or two antiviral ribozymes, each specific for a different cleavage site on the genome of the target virus, lymphocytic choriomeningitis virus (LCMV), and each contained in a self-cleavage **cassette** comprising cis-acting ribozymes designed to release the antiviral molecules from the transcript. In vitro studies showed that both antiviral ribozymes were released properly from the RNAs following cleavage by the flanking ribozymes and that these released ribozymes functioned as expected in cleaving the target virus RNA. These self-cleaving cassettes have been clones into a retroviral vector downstream of, but in the same transcript as, the chloramphenicol acetyltransferase (CAT) gene. Thus, we hoped to employ CAT as a surrogate marker of **ribozyme** transcription. Stably transformed cell lines were established. Cleavage by the cis-acting ribozymes was incomplete, as assessed by Northern blot analysis and by the ability of transformed cells to produce infectious retroviral particles. Nevertheless, the antiviral **ribozyme** sequences exerted effects in tissue culture. LCMV RNA levels in **ribozyme** -expressing cells were suppressed, and infectious virus yields were decreased by up to 95% compared with normal cells and with cells expressing inverted ribozymes. The antiviral effects correlated with CAT levels, but there was no significant difference between cell lines expressing a single ribozymes and those expressing two.

3/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08670691 96002913 PMID: 7584112
Inhibition of HIV-1 in CEM cells by a potent TAR decoy.
Lee S W; Gallardo H F; Gaspar O; Smith C; Gilboa E
Program of Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA.
Gene therapy (ENGLAND) Aug **1995**, 2 (6) p377-84, ISSN 0969-7128 Journal Code: 9421525
Contract/Grant No.: 5 R37 AI28771; AI; NIAID; K08 A10-121-01A1; PHS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

TAR decoys are short RNA oligonucleotides, corresponding to the HIV TAR sequence, which inhibit HIV expression and replication by blocking the binding of the HIV regulatory protein Tat to the authentic TAR region. In previous studies, TAR decoys expressed from a tRNA polIII promoter were moderately effective at inhibiting HIV in isolated human T cell lines and less effective at inhibiting HIV in peripheral blood CD4+ T cells. In this study, a series of modifications was introduced into the tRNA expression **cassette** in order to improve their effectiveness. These modifications included the addition of sequences which are predicted to have stem-loop

secondary structures and addition of a wild-type tRNA processing site. TAR decoy RNA expressed in CEM cells from modified tRNA-based expression cassettes yielded five- to 20-fold more TAR transcripts than unmodified tRNA-based expression cassettes. HIV replication, as measured by a flow cytometric method to quantify intracellular viral p24 expression, was significantly reduced in polyclonal populations of CEM cells expressing a modified tRNA-TAR transcript that contains a wild-type tRNA processing site and stem-loops 5' and 3' to the TAR sequence. Similar modifications to the tRNA expression **cassette** also increased the intracellular concentration of a random test oligonucleotide, indicating that this improved expression system may also be useful for antisense and **ribozyme** based gene inhibition strategies.

3/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08607741 95367477 PMID: 7640209
Altered MRP is associated with multidrug resistance and reduced drug accumulation in human SW-1573 cells.
Eijdems E W; Zaman G J; de Haas M; Versantvoort C H; Flens M J; Scheper R J; Kamst E; Borst P; Baas F
Division of Molecular Biology, The Netherlands Cancer Institute, Amsterdam.
British journal of cancer (SCOTLAND) Aug **1995**, 72 (2) p298-306
, ISSN 0007-0920 Journal Code: 0370635
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have analysed the contribution of several parameters, e.g. drug accumulation, MDR1 P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP) and topoisomerase (topo) II, to drug resistance in a large set of drug-resistant variants of the human non-small-cell lung cancer cell line SW-1573 derived by selection with low concentrations of doxorubicin or vincristine. Selection with either drug nearly always resulted in MDR clones. The resistance of these clones could be explained by reduced drug accumulation and was associated with a decrease rather than an increase in the low MDR1 mRNA level. To test whether a decrease in MDR1 mRNA indirectly affected resistance in these cells, we introduced a MDR1-specific hammerhead **ribozyme** into wild-type SW-1573 cells. Although this led to a substantial reduction in MDR1 mRNA, it did not result in resistance. In all resistant clones we found an altered form of the multidrug resistance-associated protein (MRP), migrating slightly slower during SDS-polyacrylamide gel electrophoresis than MRP in parental cells. This altered MRP was also present in non-P-gp MDR somatic cell hybrids of the SW-1573 cells, demonstrating a clear linkage with the MDR phenotype. Treatment of crude cellular membrane fractions with N-glycanase, endoglycosidase H or neuraminidase showed that the altered migration of MRP on SDS-PAGE is due to a post-translational modification. There was no detectable difference in sialic acid content. In most but not all doxorubicin-selected clones, this MDR phenotype was accompanied by a reduction in topo II alpha mRNA level. No reduction was found in the clones selected with vincristine. We conclude from these results that selection of the SW-1573 cell line for low levels of doxorubicin or vincristine resistance, predominantly results in MDR with reduced drug accumulation associated with the presence of an altered MRP protein. This mechanism can be accompanied by other resistance mechanisms, such as reduced topo II alpha mRNA in case of doxorubicin selection.

3/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08505937 95262103 PMID: 7743496
Neoplastic reversion accomplished by high efficiency adenoviral-mediated delivery of an anti-ras **ribozyme**.
Feng M; Cabrera G; Deshane J; Scanlon K J; Curiel D T
Gene Therapy Program, University of Alabama at Birmingham 35294, USA.
Cancer research (UNITED STATES) May 15 **1995**, 55 (10) p2024-8,
ISSN 0008-5472 Journal Code: 2984705R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Strategies have been developed to abrogate the aberrant expression of dominant oncogenes as a means to accomplish targeted tumor eradication. We have demonstrated previously the utility of this approach using a hammerhead **ribozyme** designed to cleave the mutant sequence in codon 12 of the activated H-ras oncogene transcript. To develop this strategy into a practical means to approach malignant disease, methods must be developed to accomplish high efficiency delivery of the **ribozyme** to target neoplastic cells. To accomplish this, a recombinant adenovirus was designed that encoded a gene **cassette** for the H-ras **ribozyme**. By using this virus, it was possible to accomplish high efficiency reversion of the neoplastic phenotype in mutant H-ras expressing tumor cells without the need for any selection steps. The demonstration of the utility of adenoviral-mediated delivery of anticancer ribozymes will allow the practical development of gene therapy strategies on this basis.

3/3,AB/14 (Item 14 from file: 155)

08339789 95098033 PMID: 7528330
Selection of efficient cleavage sites in target RNAs by using a **ribozyme** expression library.
Lieber A; Strauss M
Max-Planck-Gesellschaft zur Forderung der Wissenschaften, Humboldt University, Max Delbruck Center for Molecular Medicine, Berlin-Buch, Germany.
Molecular and cellular biology (UNITED STATES) Jan **1995**, 15 (1) p540-51, ISSN 0270-7306 Journal Code: 8109087
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Inactivation of gene expression by antisense mechanisms in general and by ribozymes in particular is a powerful technique for studying the function of a gene product. We have designed a strategy for expression of ribozymes, for selection of accessible cleavage sites in target RNAs, and for isolation of ribozymes from a library of random sequences flanking the unique sequence of a hammerhead. The expression **cassette** for **ribozyme** genes is based on adenovirus-associated RNA. Alternatively, we used polymerase III or the T7 phage transcription machinery. The **ribozyme** sequences are positioned in the center of a stable stem-loop structure, allowing for a correctly folded **ribozyme** region within the expressed RNA. A library of **ribozyme** genes with random sequences of 13 nucleotides on both sides of the hammerhead was generated. As an example, ribozymes which are specific for seven sites within the mRNA or nuclear RNA of human growth hormone were selected and identified. Sequencing of **ribozyme** genes reamplified from the library confirmed not only the predicted cleavage sites but also the presence of different **ribozyme** variants in the library. In a test of the **ribozyme** variants for repression of growth hormone synthesis in a cellular assay,

the strongest effect (more than 99% inhibition) was found for the variant with the shortest stretch of complementarity (7 and 8 nucleotides on either side) to the target RNA. This basic strategy seems to be applicable to the selection of suitable target sites and to the isolation of corresponding ribozymes for any mRNA of interest.

3/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08260861 95018229 PMID: 7932719
Interaction between tumour necrosis factor alpha **ribozyme** and cellular proteins. Involvement in **ribozyme** stability and activity.
Sioud M
Institute of Immunology and Rheumatology, Rikshospitalet, Oslo, Norway.
Journal of molecular biology (ENGLAND) Oct 7 **1994**, 242 (5) p619-29, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Ribozymes are RNA molecules that cleave other RNA molecules. Thus, ribozymes offer a new way of inhibiting expression of specific genes whose nucleotide sequences are known. Intracellular stability of ribozymes is an important factor for their efficacy. We previously showed that hammerhead **ribozyme** directed against mRNA of tumour necrosis factor alpha (TNF alpha) slowly acquires resistance to degradation in cultured human cells. In order to explain this resistance, we now report on endogenous cellular protein(s) that bind to TNF alpha-**ribozyme** (TNF alpha-Rz) in solution to form stable complexes during native gel electrophoresis. Suppression of the effects of ribonucleases in the cytoplasmic extracts allowed approximately 80% of the input **ribozyme** RNA to be recovered in the form of complexes, indicating that complex formation protected the **ribozyme** from degradation. Treatment of the **ribozyme**-protein complexes with proteinase K prior to electrophoresis led to the recovery of full-length **ribozyme**. Interestingly, **ribozyme**-protein complexes retained cleavage activity, suggesting that the binding is in reversible equilibrium. Analysis of protein cytoplasmic extracts for binding to sub-fragments of TNF alpha-Rz demonstrated that protein binds to a conformational epitope formed by an interaction between the 5' end of TNF alpha-Rz and its catalytic domain. Competition of the **ribozyme** -protein binding with a **ribozyme** construct containing DNA instead of RNA at the 5' end, indicated that the ribose phosphate backbone of the 5' end is required for strong binding. The protein responsible for the formation of the complex with low electrophoretic mobility was found to be specific for the TNF alpha-Rz, since **ribozyme** for HIV-1 integrase gene (Int-Rz), or for human interleukin-2 (IL2-Rz) did not compete significantly with the TNF alpha-Rz binding. Covalent linkage of the IL2-Rz to the 3' end of TNF alpha-Rz, or to the proposed RNA protein binding site conferred protein binding and enhanced the stability and activity of the chimeric molecules. The RNA epitope identified in this study, through its endogenous protein binding, may serve as an oligonucleotide **cassette** for enhancing the in vivo stability and activity of other RNA molecules in general. This RNA epitope will also be useful in the study of RNA-protein interactions.

3/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07882233 94020846 PMID: 8414522
Ribozyme -mediated cleavage of the BCRABL oncogene transcript: in

manner. Besides the synthetic Rz **cassette**, a comparable SalI-specific Rz **cassette** , that had been prepared from a specifically designed plasmid and that contained the tet gene inserted into the sequence of the catalytic domain of the Rz, was also incorporated into the SalI site of the PPV cDNA.(ABSTRACT TRUNCATED AT 250 WORDS)

3/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07020832 91334224 PMID: 1714571
In vivo RNA transcript-releasing plasmid possessing a universal pseudo-terminator by means of artificial ribozymes.
Ohme-Takagi M; Shinshi H; Oda M; Uchimaru T; Nishikawa S; Taira K
Fermentation Research Institute, Agency of Industrial Science & Technology, MITI, Tsukuba Science City, Japan.
Nucleic acids symposium series (ENGLAND) **1990**, (22) p49-50, ISSN 0261-3166 Journal Code: 8007206
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
RNA transcript-releasing plasmid has been constructed by means of artificial hammerhead ribozymes. In this specific construct of pGENE8459v3 the **ribozyme** targeted for SFL1 gene (a yeast suppressor gene for flocculation) was fused between two other ribozymes called 5'-processing and 3'-processing ribozymes. Since the "**Ribozyme** for SFL1" portion (**cassette**) can be replaced by other RNA sequences, it is now possible to produce any RNAs with defined 5'- and 3'- ends.

3/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06704946 91017473 PMID: 2217147
Construction of a novel artificial-**ribozyme**-releasing plasmid.
Taira K; Oda M; Shinshi H; Maeda H; Furukawa K
Fermentation Research Institute, Agency of Industrial Science and Technology, MITI, Tsukuba Science City, Fukuoka, Japan.
Protein engineering (ENGLAND) Aug **1990**, 3 (8) p733-7, ISSN 0269-2139 Journal Code: 8801484
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A novel 'active-**ribozyme**-releasing system' was constructed, taking advantage of the consensus sequence of a new class of **ribozyme**. An active **ribozyme** sequence, targeted for the SFL1 gene (a yeast suppressor gene for flocculation) was fused just downstream of the T7 promoter. The 3' terminus of the first **ribozyme** was designed to be trimmed by the second **ribozyme** connected to the downstream of the first active **ribozyme**. In vitro experiments revealed that the active **ribozyme** targeted to SFL1 was successfully released by the action of the second **ribozyme** , subsequently cleaving the SFL1 mRNA at the predetermined site. Since the first active **ribozyme** with a defined 3'-terminus can be produced even when a circular DNA is used as a template, this kind of construct has a potential to release an 'active **ribozyme** ' tailored to destroy a target gene (RNA) in vivo. Moreover, the second **ribozyme** in this construct can be utilized as a universal pseudo-terminator for generation of any RNA transcripts inserted in place of the **cassette** portion of the first **ribozyme**.

transcripts using a beta-globin 3' UTR sequence leads to an additional 15-fold increase in steady-state RNA levels. This **cassette** when used to express the best combination decoy inhibitor TAR + RRE, yields high level HIV inhibition for greater than 3 weeks. Taken together, both optimization for high level expression of molecular inhibitors and use of combinations of inhibitors suggest better therapeutic application in limiting the spread of HIV.

3/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10080726 99045584 PMID: 9826743
Recent developments in the hammerhead **ribozyme** field.
Vaish N K; Kore A R; Eckstein F
Max-Planck-Institut fur experimentelle Medizin, Hermann-Rein-Strasse 3, D-37075 Gottingen, Germany.
Nucleic acids research (ENGLAND) Dec 1 **1998**, 26 (23) p5237-42, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Developments in the hammerhead **ribozyme** field during the last two years are reviewed here. New results on the specificity of this **ribozyme** , the mechanism of its action and on the question of metal ion involvement in the cleavage reaction are discussed. To demonstrate the potential of **ribozyme** technology examples of the application of this **ribozyme** for the inhibition of gene expression in cell culture, in animals, as well as in plant models are presented. Particular emphasis is given to critical steps in the approach, including RNA site selection, delivery, vector development and **cassette** construction.

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3/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10025120 98455665 PMID: 9782345
Utilising a defective IBV RNA for heterologous gene expression with potential prophylactic application.
Evans S A; Stirrups K; Dalton K; Shaw K; Cavanagh D; Britton P
Division of Molecular Biology, Institute for Animal Health, Compton Laboratory, Newbury, Berkshire, United Kingdom.
Advances in experimental medicine and biology (UNITED STATES) **1998**, 440 p687-92, ISSN 0065-2598 Journal Code: 0121103
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Based on the natural ability of coronaviruses to undergo homologous RNA recombination, we are working to produce infectious bronchitis virus (IBV) recombinants using RNA generated from recombinant fowlpox viruses (FPV). The aim is to replace the spike (S) gene of an existing IBV vaccine strain with the S gene of a heterologous strain. CD-61 is an IBV defective RNA (D-RNA) derived from a naturally occurring IBV D-RNA (CD-91). CD-61 D-RNA is being investigated as an RNA vector for the expression of heterologous genes. T7-derived RNA transcripts of CD-61 can be replicated and passaged in the presence of helper virus, following electroporation into IBV-infected cells. CD-61 cDNA was modified by the addition of the hepatitis delta virus **ribozyme** plus T7 terminator downstream of the 3'UTR. This allowed the synthesis of discreet RNA transcripts. The complete **cassette** was cloned into an FPV transfer vector (pEFL10) for

generating recombinant fowlpox viruses. FPV/CD-61 recombinants will be assessed for D-RNA production in IBV-infected cells. The luciferase reporter gene sequence has been inserted into the modified CD-61, under the control of the IBV transcription associated sequence (TAS) from gene 5. Luciferase has been successfully expressed from CD-61 in helper virus-infected cells.

3/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09485249 97388540 PMID: 9241234
A two unit antisense RNA **cassette** test system for silencing of target genes.
Engdahl H M; Hjalt T A; Wagner E G
Department of Microbiology, Swedish University of Agricultural Sciences, Genetic Center, Box 7025, Genetikvagen 1, S-75007 Uppsala, Sweden. engdahl@mikrob.slu.se
Nucleic acids research (ENGLAND) Aug 15 **1997**, 25 (16) p3218-27
, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
This communication describes a two unit antisense RNA **cassette** system for use in gene silencing. Cassettes consist of a recognition unit and an inhibitory unit which are transcribed into a single RNA that carries sequences of non-contiguous complementarity to the chosen target RNA. The recognition unit is designed as a stem-loop for rapid formation of long-lived binding intermediates with target sequences and resembles the major stem-loop of a naturally occurring antisense RNA, CopA. The inhibitory unit consists of either a sequence complementary to a ribosome binding site or of a hairpin **ribozyme** targeted at a site within the chosen mRNA. The contributions of the individual units to inhibition was assessed using the lacI gene as a target. All possible combinations of recognition and inhibitory units were tested in either orientation. In general, inhibition of lacI expression was relatively low. Fifty per cent inhibition was obtained with the most effective of the constructs, carrying the recognition stem-loop in the antisense orientation and the inhibitory unit with an anti-RBS sequence. Several experiments were performed to assess activities of the RNAs in vitro and in vivo : antisense RNA binding assays, cleavage assays, secondary structure analysis as well as Northern blotting and primer extension analysis of antisense and target RNAs. The problems associated with this antisense RNA approach as well as its potential are discussed with respect to possible optimization strategies.

3/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09324055 97213976 PMID: 9060665
Multigene antiviral vectors inhibit diverse human immunodeficiency virus type 1 clades.
Gervaix A; Li X; Kraus G; Wong-Staal F
Department of Medicine, University of California, San Diego, La Jolla 92093-0665, USA.
Journal of virology (UNITED STATES) Apr **1997**, 71 (4) p3048-53, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: DK49618; DK; NIDDK
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed
The chronicity of infection by the human immunodeficiency virus (HIV) calls for therapeutic regimens that offer sustained antiviral effects, such as gene therapy. Recent studies have demonstrated that expression of HIV mutant transdominant proteins, RNA decoys, and ribozymes efficiently inhibited HIV replication. We have previously shown that an RNA decoy (stem-loop II of the Rev response element of HIV type 1 [HIV-1], named SL2) and a **ribozyme** (Rz) targeting the U5 region of the HIV-1 5' long terminal repeat (LTR), combined in a fusion molecule, was more efficient in inhibiting HIV-1 replication than the **ribozyme** or the decoy alone. In this study, we expressed this fusion molecule in a retrovirus-based double-copy vector to obtain higher expression of this molecule. Furthermore, we inserted a sequence internally to drive expression of another fusion molecule with a **ribozyme** targeting the env/rev region linked to SL2 to obtain a triple-copy vector. These multigene antiviral vectors were subsequently transduced or transfected into human CD4+ T cells (Molt-4). Results showed that the translocation of the SL2-Rz **cassette** from the 3' to the 5' LTR occurred in 80% of the transduced cells. The numbers of **ribozyme** RNA transcripts, estimated by competitive-quantitative reverse transcription (RT)-PCR, were 1.2 x 10(5), 1.2 x 10(4), and 1.5 x 10(3) copies per cell for the triple-copy, double-copy, and single-copy vectors, respectively. Cell challenge with multiple subtypes of HIV-1 (clades A to E) showed commensurate levels of virus inhibition for the three vectors. This study suggests that the combination of multiple anti-HIV genes, such as ribozymes and decoys, targeting multiple sites of HIV RNA and expressed at high levels are promising for the treatment of HIV-1 infection.

3/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09247669 97144634 PMID: 8990401
The expression **cassette** determines the functional activity of ribozymes in mammalian cells by controlling their intracellular localization.
Bertrand E; Castanotto D; Zhou C; Carbonnelle C; Lee N S; Good P; Chatterjee S; Grange T; Pictet R; Kohn D; Engelke D; Rossi J J
Department of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, California 91010, USA.
RNA (New York, N.Y.) (UNITED STATES) Jan **1997**, 3 (1) p75-88, ISSN 1355-8382 Journal Code: 9509184
Contract/Grant No.: AI25959; AI; NIAID; AI29329; AI; NIAID; AI33263; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In order to better understand the influence of RNA transcript context on RNA localization and catalytic RNA efficacy in vivo, we have constructed and characterized several expression cassettes useful for transcribing short RNAs with well defined 5' and 3' appended flanking sequences. These cassettes contain promoter sequences from the human U1 snRNA, U6 snRNA, or tRNA Meti genes, fused to various processing/stabilizing sequences. The levels of expression and the sub-cellular localization of the resulting RNAs were determined and compared with those obtained from Pol II promoters normally linked to mRNA production, which include a cap and polyadenylation signal. The tRNA, Ul, and U6 transcripts were nuclear in localization and expressed at the highest levels, while the standard Pol II promoted transcripts were cytoplasmic and present at lower levels. The ability of these cassettes to confer **ribozyme** activity in vivo was tested with two assays. First, an SIV-growth hormone reporter gene was transiently transfected into human embryonic kidney cells expressing an anti-SIV

vitro cleavage of RNA and in vivo loss of P210 protein-kinase activity.
Shore S K; Nabissa P M; Reddy E P
Fels Institute for Molecular Biology, Temple University School of Medicine, Philadelphia, PA 19140.
Oncogene (ENGLAND) Nov **1993**, 8 (11) p3183-8, ISSN 0950-9232 Journal Code: 8711562
Contract/Grant No.: CA 47937-01; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The t(9,22) chromosomal translocation generating the Philadelphia chromosome and the BCRABL oncogene has been shown both cytogenetically and molecularly to be the etiologic event in chronic myelogenous leukemia (CML). We have designed a **ribozyme** to cleave the BCRABL mRNA by targeting a GUU triplet adjacent to the junction of the c-BCR and c-ABL fused genes. This **ribozyme** efficiently cleaved BCRABL RNA transcripts as demonstrated by in vitro cleavage reactions. To determine the effect of constitutive expression of the **ribozyme** on the elimination of the BCRABL gene product, the **ribozyme** cDNA sequence was inserted into different retroviral expression vectors. Introduction of the recombinant retroviruses into the CML blast crisis cell-line K562, resulted in the elimination of the P210 protein-kinase activity in several single cell clones infected with the **ribozyme** expression **cassette** . Therefore BCR-ABL specific ribozymes may provide a potential genetic therapy for CML.

3/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07150773 92084134 PMID: 1660835
Catalytic antisense RNAs produced by incorporating **ribozyme** cassettes into cDNA.
Tabler M; Tsagris M
Foundation for Research and Technology, Institute of Molecular Biology and Biotechnology, Heraklion/Crete, Greece.
Gene (NETHERLANDS) Dec 15 **1991**, 108 (2) p175-83, ISSN 0378-1119 Journal Code: 7706761
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

A simple strategy is described for the generation of catalytic hammerhead-type ribozymes (Rz) that can be used as highly specific endoribonucleases to cleave a particular target RNA. The technique requires that a cloned cDNA fragment is available which encodes at least a part of the target RNA. About 25 different restriction recognition sequences can be utilized to incorporate specifically designed DNA cassettes into the cDNA. Besides some nucleotides which are specific for a certain restriction site, the DNA cassettes contain a sequence corresponding to the catalytic domain of the hammerhead Rz and, optionally, selectable marker genes, that are removable. The resulting recombinant DNA constructs permit the in vitro and in vivo synthesis of novel 'catalytic antisense RNAs' or 'antisense Rz (Az)', which combine two features: (i) they bind like antisense RNA to their specific substrate RNA, and (ii) they cleave their target as hammerhead Rz do. The utility of the strategy to generate Rz was demonstrated experimentally by incorporating a synthetic SalI-specific DNA **ribozyme** (Rz) **cassette** into a unique SalI site of a cloned cDNA fragment of plum pox virus (PPV), which is a single-stranded positive sense plant RNA virus, belonging to the group of potyviruses. The resulting Az constructs delivered Az that were directed against the PPV (+) or (-) RNA, respectively, which cleaved their corresponding target RNAs in the expected

Lieber A; Kay M A
Markey Molecular Medicine Center, Division of Medical Genetics, Department of Medicine, University of Washington, Seattle 98195, USA.
Journal of virology (UNITED STATES) May **1996**, 70 (5) p3153-8, ISSN 0022-538X Journal Code: 0113724
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Ribozymes are a new pharmaceutical class of reagents that offer potential in treating a number of different medical disorders, including infectious diseases and cancer. As a first step towards using ribozymes for the treatment of liver disorders such as viral hepatitis, adenovirus vectors that contain a **ribozyme** expression **cassette** under the control of different promoters directed against human growth hormone (hGH) were constructed and infused into transgenic mice that produce hGH from the gastrointestinal tract and liver. Adenovirus-mediated transfer of expressed ribozymes resulted in up to a 96% reduction of hepatic hGH mRNA over a period of several weeks in the transgenic mouse model. Furthermore, the concentration of **ribozyme** RNA correlated with the degree of hGH mRNA reduction. This study clearly demonstrates that ribozymes can function during the period of expression in an intact organ after somatic gene transfer.

3/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08842883 96187812 PMID: 8614996
Intracellular immunization against SIVmac utilizing a hairpin **ribozyme**.
Heusch M; Kraus G; Johnson P; Wong-Staal F
Department of Biology, University of California at San Diego, La Jolla 92093-0665, USA.
Virology (UNITED STATES) Feb 1 **1996**, 216 (1) p241-4, ISSN 0042-6822 Journal Code: 0110674
Contract/Grant No.: DK49618; DK; NIDDK
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A hairpin **ribozyme** targeting the 3' LTR region (9456) of SIVmac238 was cloned into a murine retroviral vector. This target sequence is conserved among various SIV, as well as most HIV-2, strains. The **ribozyme cassette** is driven from a polymerase III promoter, that of the human tRNAval gene. Hybrid human B-/T-cell lines (CEM/174) were transduced with the retroviral constructs and selected for G418 resistance. Cells stably expressing the 9456 **ribozyme** exhibited long-term resistance to infection by a pathogenic molecular clone of SIV and two strains of HIV-2. The **ribozyme** was also able to effectively reduce the proviral DNA burden. Its efficient protection against SIV/HIV-2 infection constitutes an important step toward evaluating **ribozyme** gene therapy in a primate model.

3/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08766185 96113286 PMID: 8785476
Antiviral activity of RNA molecules containing self-releasing ribozymes targeted to lymphocytic choriomeningitis virus.
Xing Z; Mahadeviah S; Whitton J L

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     $0.00  Estimated total session cost   0.214 DialUnits

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         (c) 2003 BIOSIS
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removal, customized scheduling. See HELP ALERT.

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          21931987  PY<1999
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           21896  HBV
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          153308  VECTOR
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 3/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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14371692   22403562   PMID: 12515183
  Synthesis and preliminary study on cleavage activity of ribozyme to
hepatitis B virus preS2 gene in vitro]
  Xu D; Han F; Shi H; et al
  Biological Engineering Laboratory of 302 Hospital, Beijing 100039.
  Zhonghua  shi  yan  he lin chuang bing du xue za zhi = Zhonghua shiyan he
linchuang  bingduxue  zazhi  = Chinese journal of experimental and clinical
virology (China)   Jun 1998,  12  (2)  p107-10,  ISSN 1003-9279
Journal Code: 9602873
  Document type: Journal Article ; English Abstract
  Languages: CHINESE
  Main Citation Owner: NLM
  Record type: Completed
  In  this  paper,  a  multi-target  hammerhead  ribozyme  gene  was
synthesized directed against 110,  122  and  132  sites of nucleotide of
HBV  preS2  gene.  The  target  gene  fragment was cut from HBV
 genome containing plasmid pCP10. Both of the ribozyme and the target
gene fragments were cloned into pGEM3Zf(-) plasmid and sequenced by dideoxy
chain  termination  method.  The  transcription  of both gene fragments was
performed  in  vitro  utilizing T7 RNA promoter in pGEM3Zf (-) plasmid. The
cleavage  activity  of  ribozyme to substrate was confirmed in vitro.
For  further  evaluating  intracellular  function  of  ribozyme,  two
ribozyme-retroviral recombinant plasmids with different promoter type
pDOR-ripe  and  tRNA-ripe  were  constructed.  Pseudo-virus  was  collected
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through routine packaging procedure and transduted into 2.2.15 cells. RIA data showed a stable inhibition of pHSA-R antigen expression to the lowest extent of 41.01% +/- 4.16 and the highest extent of 51.45% +/- 4.57 within 4 weeks after transduction. No influence, however, on HBsAg and HBeAg expression was demonstrated after **ribozyme** gene transfer.

3/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09531004 97427055 PMID: 9282175
Intracellular application of hairpin **ribozyme** genes against hepatitis B virus.
Welch P J; Tritz R; Yei S; Barber J; Yu M
Immusol, Inc., San Diego, CA 92121, USA.
Gene therapy (ENGLAND) Jul **1997**, 4 (7) p736-43, ISSN 0969-7128 Journal Code: 9421525
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
HBV , a partially double-stranded DNA virus, replicates through a pregenomic RNA (pgRNA) intermediate, which provides a therapeutic opportunity for a novel antiviral gene therapy based on **ribozyme** RNA cleavage. Three hairpin ribozymes (Rzs) were designed which have the potential to disrupt **HBV** replication by targeting the pgRNA as well as specific mRNAs encoding the **HBV** surface antigen (HBsAg), the polymerase and the X protein. The ability of each **ribozyme** to cleave approximately 0.3 kb **HBV** subgenomic RNA fragments was tested in vitro. Two of the three Rzs tested (BR1 and BR3) were capable of cleaving their respective RNA substrates, while their catalytically disabled mutated counterpart Rzs were not. Structural modifications were performed on these two Rzs, with the goal of increasing catalytic efficiency both in vitro and in cells. To determine the Rz activities in liver cells, the cDNAs for each of the anti-**HBV** Rzs (and their catalytically disabled negative controls) were cloned into retroviral vectors. Unmodified ribozymes co-expressed with **HBV** in human liver Huh7 cells reduced the level of viral particle production by up to 66% based on the endogenous polymerase assay, while the structurally modified ribozymes inhibited **HBV** production up to 83%. These encouraging results indicate the feasibility of **ribozyme**-mediated gene therapy for the treatment of **HBV** infections.

3/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09303446 97196618 PMID: 9043708
Design and preparation of a multimeric self-cleaving hammerhead **ribozyme**.
Ruiz J; Wu C H; Ito Y; Wu G Y
University of Connecticut Health Center, Farmington, USA.
BioTechniques (UNITED STATES) Feb **1997**, 22 (2) p338-45, ISSN 0736-6205 Journal Code: 8306785
Contract/Grant No.: DK-42182; DK; NIDDK
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The activity of a **ribozyme** can be impaired by additional sequences at the 5' and 3' termini of the catalytic sequence. To approach this problem, a system was designed that minimizes sequences upstream and

downstream from active regions of a hammerhead **ribozyme** and allows delivery of a large number of active molecules. A self-cleavable multimeric molecule was prepared by placing a **ribozyme** target sequence (derived from the core region of the hepatitis B virus [**HBV**]) upstream and downstream from the catalytic sequence. This construct was cloned in tandem into in vitro expression vectors. 32P-UTP-labeled transcripts of the multimeric construct, as well as non-self-cleaved monomeric **ribozyme** controls, and substrate were synthesized. The multimeric **ribozyme** molecule efficiently self-cleaved to release monomeric ribozymes lacking any extra upstream and downstream sequences. In addition, monomers were substantially more active against the **HBV** target RNA than the non-self-cleavable ribozymes. Up to 80% degradation of the target RNA was achieved by a tenfold molar excess of a pentameric construct. We conclude that ribozymes can be produced as a multimeric tandem of self-cleavable molecules, the monomers of which are more active than monomeric ribozymes and highly efficient in cleavage of target transcripts.

3/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09024828 96384185 PMID: 8792082
Hepatitis delta virus. Genetics and pathogenesis.
Casey J L
Division of Molecular Virology and Immunology, Georgetown University Medical Center, Washington, DC, USA.
Clinics in laboratory medicine (UNITED STATES) Jun **1996**, 16 (2) p451-64, ISSN 0272-2712 Journal Code: 8100174
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
After the discovery of HDV there have been significant advances in the understanding of the biology and disease of HDV infection. Analyses at the molecular level have revealed several fascinating features (**ribozyme** activity, RNA-dependent RNA polymerase activity of RNA polymerase II, HDAg isoprenylation, and RNA editing) that are of significant interest. Intensive investigation of the **ribozyme** elements has yielded important insights in both functional and structural features. However, there is information lacking about other aspects of the HDV replication cycle including the specific nature of the interaction between HDAg and HDV RNA, the function of HDAg in HDV RNA replication, transcription by RNA polymerase II, and the mechanisms of HDV RNA editing and its regulation. Further study of these and other aspects of the HDV replication cycle will continue to enrich our understanding of basic biology. Evaluation of the mechanisms of HDV disease remains an important goal in the study of this agent. Although both acute and chronic disease are commonly associated with unfavorable outcomes, it is clear that chronic infection is associated with a broad spectrum of disease. The interactions between HDV, **HBV**, and the host are necessarily complex, and it is likely that each contribute factors that influence disease and outcome. Recent analyses of HDV genotypes have suggested that disease variations may be associated with viral genetic factors. Consistent with the obligate role of **HBV** in the HDV life cycle, **HBV** replication is also an important determinant of HDV disease. It is still unclear if interactions between specific genotypes or variants of **HBV** and HDV influence disease. Recent data also suggest that infection with multiple hepatitis viruses (**HBV**, HDV, and HCV) can influence the severity of disease. It remains to be seen whether coinfection with the recently discovered hepatitis G virus is associated with altered disease patterns. Further advances in our understanding HDV disease and possible therapeutic approaches will rely on a combination of additional studies at the molecular, genetic, epidemiologic, and clinical levels. These studies will continue to

elaborate the model of HDV infection and disease that can ultimately be tested by experimental infection of chimpanzees and woodchucks.

3/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08782688 96145250 PMID: 8559651
Efficient hammerhead **ribozyme**-mediated cleavage of the structured hepatitis B virus encapsidation signal in vitro and in cell extracts, but not in intact cells.
Beck J; Nassal M
Zentrum fur Molekulare Biologie, Universitat Heidelberg, Germany.
Nucleic acids research (ENGLAND) Dec 25 **1995**, 23 (24) p4954-62
, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Hepatitis B virus (**HBV**), the causative agent of B-type hepatitis in man, is a small enveloped DNA virus that replicates through reverse transcription of an RNA intermediate, the terminally redundant RNA pregenome. An essential highly conserved cis-element present twice on this RNA is the encapsidation signal epsilon, a stem-loop structure that is critical for pregenome packaging and reverse transcription. Epsilon is hence an attractive target for antiviral therapy. Its structure, however, is a potential obstacle to antivirals whose action depends on hybridization, e.g. ribozymes. Here we demonstrate effective in vitro cleavage inside epsilon by hammerhead ribozymes containing flanking sequences complementary to an adjacent less structured region. Upon co-transfection with a **HBV** expression construct corresponding ribozymes embedded in a U6 snRNA context led to a significant, though modest, reduction in the steady-state level of **HBV** pregenomes. Inactive **ribozyme** mutants revealed that antisense effects contributed substantially to this reduction, however, efficient epsilon cleavage by the intracellularly expressed ribozymes was observed in Mg(2+)-supplemented cell lysates. Artificial **HBV** pregenomes carrying the ribozymes in cis and model RNAs lacking all **HBV** sequences except epsilon exhibited essentially the same behaviour. Hence, neither the absence of co-localization of **ribozyme** and target nor a viral component, but rather a cellular factor(s), is responsible for the strikingly different **ribozyme** activities inside cells and in cellular extracts.

3/3,AB/6 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11454953 BIOSIS NO.: 199800236285
Hepatitis D virus.
AUTHOR: Karayiannis P(a)
AUTHOR ADDRESS: (a)Dep. Med., Div. Med., Imperial Coll. Sch. Med. St. Mary's, South Wharf Road, London W2 1NY**UK
JOURNAL: Reviews in Medical Virology 8 (1):p13-24 Jan.-March, **1998**
ISSN: 1052-9276
DOCUMENT TYPE: Literature Review
RECORD TYPE: Citation
LANGUAGE: English
1998

3/3,AB/7 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10224058 BIOSIS NO.: 199698678976
Putative secondary structure of human hepatitis B viral X mRNA.
AUTHOR: Kim Hadong; Choi Yoon Chul; Lee Bum Yong; Junn Eunsung; Ahn Jeongkeun; Kang Changwon; Park Inwon(a)
AUTHOR ADDRESS: (a)Dep. Chemistry, Seoul National University, Seoul 151-742 **South Korea
JOURNAL: Journal of Biochemistry and Molecular Biology 28 (6):p509-514 **1995**
ISSN: 1225-8687
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A putative secondary structure of the mRNA for the human hepatitis B virus (**HBV**) X gene is proposed based on not only chemical and enzymatic determination of its single- and double-stranded regions but also selection by the computer program MFOLD for energy minimum conformation under the constraints that the experimentally determined nucleotides were forced or prohibited to base pair. An RNA of 536 nucleotides including the 461-nucleotide **HBV** X mRNA sequence was synthesized in vitro by the phage T7 RNA polymerase transcription. The thermally renatured transcripts were subjected to chemical modifications with dimethylsulfate and kethoxal and enzymatic hydrolysis with single strand-specific RNase T1 and double strand-specific RNase V1, separately. The sites of modification and cleavage were detected by reverse transcriptase extension of 4 different primers. Many nucleotides could be assigned with high confidence, twenty in double-stranded and thirty-seven in single-stranded regions. These nucleotides were forced and prohibited, respectively, to base pair in running the recursive RNA folding program MFOLD. The results suggest that 6 different regions (5 within X mRNA) of 14 apprx 23 nucleotides are single-stranded. This putative structure provides a good working model and suggests potential target sites for antisense and **ribozyme** inhibitors and hybridization probes for the **HBV** X mRNA.

1995

3/3,AB/8 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10174202 BIOSIS NO.: 199698629120
Therapy of hepadnavirus infection using antisense oligonucleotides.
AUTHOR: Offensperger Wolf-Bernhard(a); Blum Hubert E; Gerok Wolfgang
AUTHOR ADDRESS: (a)Dep. Med., Univ. Freiburg, D-79106, Freiburg**Germany
JOURNAL: Intervirology 38 (1-2):p113-119 **1995**
ISSN: 0300-5526
DOCUMENT TYPE: Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Chronic infection with the hepatitis B virus (**HBV**) is a major health problem worldwide. The only established therapy is alpha-interferon with an efficacy of only 30-40% in highly selected patients. Major theoretical problems of therapeutical strategies against hepadnaviral infections are the limited immune response and the presence of covalently closed **HBV** DNA in the nucleus. Many nucleoside analogues and inhibitors of viral reverse transcriptases were tested in vitro and in vivo with transient effects and often severe side effects. Molecular therapeutic strategies include antisense DNA/RNA and ribozymes. In vitro antisense oligodeoxynucleotides could be shown to inhibit viral

replication and gene expression in human hepatoma cell lines. In vivo an antisense oligodeoxynucleotide directed against the 5'-region of the preS gene of the duck hepatitis B virus inhibited the viral replication and gene expression in ducks. These results demonstrate the potential clinical use of antisense DNA/RNA as antiviral therapeutics.

1995

3/3,AB/9 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10082997 BIOSIS NO.: 199598537915
Study on the cDNA synthesis and gene clone of multitarget-**ribozyme** directed to cleave the three sites of S region of hepatitis B virus (**HBV**) pregenomic RNA in vitro.
AUTHOR: Wang F S; Han F L; Jin L; Shi H; Lei Z Y
AUTHOR ADDRESS: Dep. Bioeng., Inst. Infect. Dis., 26 Feng Tai Road, Beijing 100039**China
JOURNAL: Hepatology 22 (4 PART 2):p507A **1995**
CONFERENCE/MEETING: 46th Annual Meeting and Postgraduate Course of the American Association for the Study of Liver Diseases Chicago, Illinois, USA November 3-7, 1995
ISSN: 0270-9139
RECORD TYPE: Citation
LANGUAGE: English
1995

3/3,AB/10 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09767403 BIOSIS NO.: 199598222321
Molecular biologic and pathogenetic analysis of hepatitis delta virus.
AUTHOR: Lai Michael M C
AUTHOR ADDRESS: Howard Hughes Med. Inst., Univ. Southern California, Sch. Med., Los Angeles, CA 90033**USA
JOURNAL: Journal of Hepatology 22 (SUPPL. 1):p127-131 **1995**
ISSN: 0168-8278
DOCUMENT TYPE: Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Hepatitis delta virus (HDV) consists of an envelope derived from hepatitis B virus (**HBV**) and a nucleocapsid consisting of hepatitis delta antigen and a circular RNA genome. Both the delta antigen and the RNA possess many unique properties which play crucial roles in the life cycle of HDV. They may also contribute indirectly to the mechanism of viral pathogenesis.

1995

3/3,AB/11 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08613415 BIOSIS NO.: 199345031490
Effects of **ribozyme** on **HBV** in vitro and in HepG2 cell.
AUTHOR: Wang Ping; Guo Jutao; Xu Wei; Su Hue; Zeng Liyu; Hou Yunde
AUTHOR ADDRESS: Inst. Virol., Beijing 100052**China
JOURNAL: FASEB Journal 7 (7):pA1301 **1993**

CONFERENCE/MEETING: Joint Meeting of the American Society for Biochemistry and Molecular Biology and American Chemical Society Division of Biological Chemistry San Diego, California, USA May 30-June 3, 1993
ISSN: 0892-6638
RECORD TYPE: Citation
LANGUAGE: English
1993

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              6  CHPBI
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           6181  SOC
           6548  SOS
              6  SRNC
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              8  PNDB
            664  SOF
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DIALOG(R)File 155:MEDLINE(R)

10102560 99096459 PMID: 9878787
Expression pattern and cellular distribution of the murine homologue of AF10.
Linder B; Jones L K; Chaplin T; Mohd-Sarip A; Heinlein U A; Young B D; Saha V
The Imperial Cancer Research Fund, Department of Medical Oncology, Charterhouse Square, St Bartholomew's and the Royal London Hospital School of Medicine, London EC1M 6BQ, UK.linder@icrf.icnet.uk
Biochimica et biophysica acta (NETHERLANDS) Dec 22 **1998**, 1443 (3) p285-96, ISSN 0006-3002 Journal Code: 0217513
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have cloned Af10, the murine homologue of the MLL partner gene AF10. The predicted open reading frame of Af10 contains 1069 aa which are 90% identical to those of AF10. Af10 contains an N-terminal cysteine-rich region with a LAP/**PHD** finger, a leucine zipper domain and a glutamine-rich region at the C-terminus, features also found in the human proteins AF10 and AF17. A single 5. 5-kb transcript was detected in murine tissues with the highest level of expression in the testes. A polyclonal antibody raised to the cysteine-rich region of AF10 was able to identify a double band of 140 kDa on Western analysis in mouse testicular extracts. After subcellular separation Af10 was identified in both the nuclear and cytoplasmic extracts, again as a double band of 140 kDa in size. In situ hybridisation studies were performed with sense and **antisense** digoxigenin-labelled oligonucleotides. High levels of expression were noted in postmeiotic germ cells, especially in spermatids from around stage VI to stage VIII. High levels of expression were also seen in the white matter of the cerebellum, extending into the granular layer. The expression in differentiated rather than in proliferating cells suggests that the role of Af10 may lie in the suppression of proliferation rather than in differentiation. Since the LAP/**PHD** finger domains are lost in the MLL-AF10 fusion, arguably such a function could be carried out by this domain.

3/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09967761 98401411 PMID: 9726918
Structural studies of a stable parallel-stranded DNA duplex incorporating isoguanine:cytosine and isocytosine:guanine basepairs by nuclear magnetic resonance spectroscopy.
Yang X L; Sugiyama H; Ikeda S; Saito I; Wang A H
Department of Cell and Structural Biology, University of Illinois, Urbana-Champaign 61801, USA.
Biophysical journal (UNITED STATES) Sep **1998**, 75 (3) p1163-71, ISSN 0006-3495 Journal Code: 0370626
Contract/Grant No.: GM41612; GM; NIGMS; S104406243; PHS; S10RR10444-01; RR; NCRR
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Isoguanine (2-hydroxyladenine) is a product of oxidative damage to DNA and has been shown to cause mutation. It is also a potent inducer of parallel-stranded DNA duplex structure. The structure of the parallel-stranded DNA duplex (PS-duplex) 5'-d(TiGiCAiCiGiGAiCT) + 5'-d(ACGTGCCTGA), containing the isoguanine (iG) and 5-methyl-isocytosine

(iC) bases, has been determined by NMR refinement. All imino protons associated with the iG:C, G:iC, and A:T (except the two terminal A:T) basepairs are observed at 2 degrees C, consistent with the formation of a stable duplex suggested by the earlier Tm measurements [Sugiyama, H., S. Ikeda, and I. Saito. 1996. J. Am. Chem. **Soc**. 118:9994-9995]. All basepairs are in the reverse Watson-Crick configuration. The structural characteristics of the refined PS-duplex are different from those of B-DNA. The PS duplex has two grooves with similar width (7.0 A) and depth (7.7 A), in contrast to the two distinct grooves (major groove width 11.7 A, depth 8.5 A, and minor groove width 5.7 A, depth 7.5 A) of B-DNA. The resonances of the amino protons of iG and C are clearly resolved and observable, but those of the G and iC are very broad and difficult to observe. Several intercalators with different complexities, including ethidium, daunorubicin, and nogalamycin, have been used to probe the flexibility of the backbone of the (iG, iC)-containing PS-duplex. All of them produce drug-induced UV/vis spectra identical to their respective spectra when bound to B-DNA, suggesting that those drugs bind to the (iG, iC)-containing PS-duplex using similar intercalation processes. The results may be useful in the design of intercalator-conjugated oligonucleotides for **antisense** applications. The study presented in this paper augments our understanding of a growing number of parallel-stranded DNA structures, including the G-quartet, the i-motif, and the unusual homo basepaired parallel-stranded double helix. Their possible relevance is discussed.

3/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09909461 98316914 PMID: 9654342
Intracellular Ca2+ store-operated influx of Ca2+ through TRP-R, a rat homolog of TRP, expressed in Xenopus oocytes.
Tomita Y; Kaneko S; Funayama M; Kondo H; Satoh M; Akaike A
Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Kyoto University, Japan.
Neuroscience letters (IRELAND) Jun 5 **1998**, 248 (3) p195-8, ISSN 0304-3940 Journal Code: 7600130
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
To elucidate whether rat transient receptor potential (TRP-R), a rat TRP4 homolog, functions as a store-operated Ca2+ channel (**SOC**), we have measured the Ca2+ entry after thapsigargin treatment in Xenopus oocytes injected with mRNA for TRP-R. While non-injected oocytes elicited an **SOC** response, significantly larger responses were observed in the oocytes expressing TRP-R. The oocyte-native **SOC** response was inhibited by injection of **antisense** oligodeoxyribonucleotide for mammalian TRP1. When Ca2+ concentration-**SOC** response curve was examined, the EC50 value was much smaller in oocytes expressing TRP-R than that of non-injected oocytes. These results suggest that TRP-R functions as **SOC** having higher sensitivity to external Ca2+ than amphibian TRP1 channel.

3/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09892134 98332535 PMID: 9665717
Unusual metal ion catalysis in an acyl-transferase **ribozyme**.
Suga H; Cowan J A; Szostak J W
Department of Molecular Biology, Massachusetts General Hospital, Boston 02114, USA. hsuga@acsu.buffalo.edu

Biochemistry (UNITED STATES) Jul 14 **1998**, 37 (28) p10118-25, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: R01 GM53936; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Most studies of the roles of catalytic metal ions in **ribozymes** have focused on inner-sphere coordination of the divalent metal ions to the substrate or **ribozyme** . However, divalent metal ions are strongly hydrated in water, and some proteinenzymes, such as Escherichia coli RNase H and exonuclease III, are known to use metal cofactors in their fully hydrated form [Duffy, T. H., and Nowak, T. (1985) Biochemistry 24, 1152-1160; Jou, R., and Cowan, J. A. (1991) J. Am. Chem. **Soc**. 113, 6685-6686]. It is therefore important to consider the possibility of outer-sphere coordination of catalytic metal ions in **ribozymes**. We have used an exchange-inert metal complex, cobalt hexaammine, to show that the catalytic metal ion in an acyl-transferase **ribozyme** acts through outer-sphere coordination. Our studies provide an example of a fully hydrated Mg2+ ion that plays an essential role in **ribozyme** catalysis. Kinetic studies of wild-type and mutant **ribozymes** suggest that a pair of tandem G:U wobble base pairs adjacent to the reactive center constitute the metal-binding site. This result is consistent with recent crystallographic studies [Cate, J. H., and Doudna, J. A. (1996) Structure 4, 1221-1229; Cate, J. H., Gooding, A. R., Podell, E., Zhou, K., Golden, B. L., Kundrot, C. E., Cech, T. R., and Doudna, J. A. (1996) Science 273, 1678-1685; Cate, J. H., Hanna, R. L., and Doudna, J. A. (1997) Nat. Struct. Biol. 4, 553-558] showing that tandem wobble base pairs are good binding sites for metal hexaammines. We propose a model in which the catalytic metal ion is bound in the major groove of the tandem wobble base pairs, is precisely positioned by the **ribozyme** within the active site, and stabilizes the developing oxyanion in the transition state. Our results may have significant implications for understanding the mechanism of protein synthesis [Noller, H. F., Hoffarth, V., and Zimniak, L. (1992) Science 256, 1416-1419].

3/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09805408 98238634 PMID: 9571148

In vitro and in vivo potentiation of radiosensitivity of malignant gliomas by **antisense** inhibition of the RAD51 gene.

Ohnishi T; Taki T; Hiraga S; Arita N; Morita T
Department of Neurosurgery, Osaka University Medical School, Japan. ohnishi@nsurg.med.osaka-u.ac.jp

Biochemical and biophysical research communications (UNITED STATES) Apr 17 **1998**, 245 (2) p319-24, ISSN 0006-291X Journal Code: 0372516
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The mammalian RAD51 gene is a homologue of the yeast RAD51 and E. coli RecA genes, which are related to the repair of DNA double-strand breaks and are also involved in recombination repair and various **SOS** responses to DNA damage by gamma-irradiation and alkylating reagents. In this study, we investigated both in vitro and in vivo whether inhibition of the RAD51 gene by **antisense** oligonucleotides (ODNs) enhances the radiosensitivity of mouse malignant gliomas. A volume of 100 nM of RAD51 **antisense** ODNs inhibited the level of mRNA by more than 95% and reduced the protein expression by about 70%. Treatment of mouse 203G glioma cells with 100 nM of RAD51 **antisense** ODNs significantly enhanced the radiation-induced cell kill compared to control cells, and cells treated

with sense or scrambled ODNs. When the glioma cells were implanted in the cisterna magna of mice followed by treatment with RAD51 **antisense** ODNs, the survival time of the mice was markedly prolonged compared to that of the untreated group ($p < 0.001$, logrank test). In addition, the combination of **antisense** ODNs and irradiation extended the survival time of the glioma-bearing mice much longer than could be achieved with radiation alone ($p < 0.0001$, logrank test). These results suggest that inhibition of RAD51 can be expected to serve as a novel potentiator for radiation therapy in malignant gliomas by inhibiting DNA double-strand break repair.

3/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09631859 98062208 PMID: 9401036
Evidence of participation of McrB(S) in McrBC restriction in Escherichia coli K-12.
Beary T P; Braymer H D; Achberger E C
Department of Biological Sciences, Nicholls State University, Thibodaux, Louisiana 70310, USA.
Journal of bacteriology (UNITED STATES) Dec **1997**, 179 (24) p7768-75, ISSN 0021-9193 Journal Code: 2985120R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The McrBC restriction system has the ability to restrict DNA containing 5-hydroxymethylcytosine, N4-methylcytosine, and 5-methylcytosine at specific sequences. The mcrB gene produces two gene products. The complete mcrB open reading frame produces a 51-kDa protein (McrB(L)) and a 33-kDa protein (McrB(S)). The smaller McrB polypeptide is produced from an in-frame, internal translational start site in the mcrB gene. The McrB(S) sequence is identical to that of McrB(L) except that it lacks 161 amino acids present at the N-terminal end of the latter protein. It has been suggested that McrB(L) is the DNA binding restriction subunit. The function of McrB(S) is unknown, although there has been speculation that it plays some role in the modulation of McrBC restriction. Studies of the function of McrB(S) have been challenging since it is produced in frame with McrB(L). In this study, we tested the effects of underproduction (via **antisense** RNA) and overproduction (via gene dosage) of mcrBC gene products on restriction levels of the mcrBC+ strain JM107. Among the parameters monitored was the induction of **SOS** responses, which indicate of DNA damage. Evidence from this study suggests that McrB(S) is necessary for stabilization of the McrBC restriction complex in vivo.

3/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09508794 97415597 PMID: 9271396
Requirement of protein kinase C zeta for stimulation of protein synthesis by insulin.
Mendez R; Kollmorgen G; White M F; Rhoads R E
Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, Shreveport 71130, USA.
Molecular and cellular biology (UNITED STATES) Sep **1997**, 17 (9) p5184-92, ISSN 0270-7306 Journal Code: 8109087
Contract/Grant No.: DK 38712; DK; NIDDK; DK 43808; DK; NIDDK; GM 20818; GM; NIGMS; +
Document type: Journal Article
Languages: ENGLISH

Main Citation Owner: NLM
Record type: Completed

The ability of insulin to stimulate protein synthesis and cellular growth is mediated through the insulin receptor (IR), which phosphorylates Tyr residues in the insulin receptor substrate-signaling proteins (IRS-1 and IRS-2), Gab-1, and Shc. These phosphorylated substrates directly bind and activate enzymes such as phosphatidylinositol 3'-kinase (PI3K) and the guanine nucleotide exchange factor for p21Ras (GRB-2/**SOS**), which are in turn required for insulin-stimulated protein synthesis, cell cycle progression, and prevention of apoptosis. We have now shown that one or more members of the atypical protein kinase C group, as exemplified by the zeta isoform (PKC zeta), are downstream of IRS-1 and P13K and mediate the effect of insulin on general protein synthesis. Ectopic expression of constitutively activated PKC zeta eliminates the requirement of IRS-1 for general protein synthesis but not for insulin-stimulated activation of 70-kDa S6 kinase (p70S6K), synthesis of growth-regulated proteins (e.g., c-Myc), or mitogenesis. The fact that PKC zeta stimulates general protein synthesis but not activation of p70S6K indicates that PKC zeta activation does not involve the proto-oncogene Akt, which is also activated by PI3K. Yet insulin is still required for the stimulation of general protein synthesis in the presence of constitutively active PKC zeta and in the absence of IRS-1, suggesting a requirement for the convergence of the IRS-1/PI3K/PKC zeta pathway with one or more additional pathways emanating from the IR, e.g., Shc/**SOS**/p21Ras/mitogen-activated protein kinase. Thus, PI3K appears to represent a bifurcation in the insulin signaling pathway, one branch leading through PKC zeta to general protein synthesis and one, through Akt and the target of rapamycin (mTOR), to growth-regulated protein synthesis and cell cycle progression.

3/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09386127 97288082 PMID: 9143123

Combining the hok/sok, parDE, and pnd postsegregational killer loci to enhance plasmid stability.

Pecota D C; Kim C S; Wu K; Gerdes K; Wood T K

Department of Chemical and Biochemical Engineering, University of California, Irvine 92697-2575, USA.

Applied and environmental microbiology (UNITED STATES) May **1997**, 63 (5) p1917-24, ISSN 0099-2240 Journal Code: 7605801

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

To enhance plasmid segregational stability in bacterial cells, two pairs of independent postsegregational killing loci (genes which induce host killing upon plasmid loss) isolated from plasmids R1, R483, or RP4 (hok+/sok+ pnd+ or hok+/sok+ parDE+) were cloned into a common site of the beta-galactosidase expression vector pMJR1750 (ptac::lacZ+) to form a series of plasmids in which the effect of one or two stability loci on segregational plasmid stability could be discerned. Adding two **antisense** killer loci (hok+/sok+ pnd+) decreased the specific growth rate by 50% though they were more effective at reducing segregational instability than hok+/sok+ alone. With the ptac promoter induced fully (2.0 mM isopropyl-beta-D-thiogalactopyranoside) and no antibiotic selection pressure, the combination of a proteic killer locus (parDE+) with **antisense** killer loci (hok+/sok+) had a negligible impact on specific growth rate, maintained high beta-galactosidase expression, and led to a 30 and 190% increase in segregational stability (based on stable generations) as compared to plasmids containing either hok+/sok+ or parDE+ alone, respectively. Use of hok+/sok+ or parDE+ alone with high cloned-gene expression led to ninefold and fourfold increases in the number of stable

generations, respectively. Two convenient cloning cassettes have been constructed to facilitate cloning the dual hok+/sok+ parDE+ and hok+/sok+ pnd+ killer systems.

3/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09349492 97251006 PMID: 9096689
Antisense src expression inhibits tyrosine phosphorylation of Shc and its association with Grb2 and **Sos** which leads to MAP kinase activation in U937 human leukemia cells.
Yamaguchi M; Tanaka T; Waki M; Kitanaka A; Kamano H; Kubota Y; Ohnishi H; Takahara J; Irino S
First Department of Internal Medicine, Kagawa Medical School, Japan.
Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K (ENGLAND) Apr **1997**, 11 (4) p497-503, ISSN 0887-6924 Journal Code: 8704895
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We constructed a recombinant plasmid which expresses **antisense** src RNA after dexamethasone (Dexa) treatment, and transfected it into U937 human monoblastic leukemia cells (U937-ASRC). Induction of **antisense** src RNA expression diminished the amounts of c-Src and its protein tyrosine kinase (PTK) activity in U937-ASRC cells. The declines in c-Src and its PTK activity subsequently reduced the proliferation of U937-ASRC cells. To elucidate the growth signal transduction pathway downstream of c-Src, tyrosine phosphorylation of Shc was examined in U937-ASRC cells treated with Dexa. The decline in c-Src by induction of **antisense** src RNA expression decreased the level of tyrosine phosphorylation of Shc. Immunoprecipitated c-Src directly phosphorylated immunoprecipitated Shc on tyrosine residues in vitro. The amounts of Grb2 and **Sos** co-immunoprecipitated with Shc were decreased after Dexa treatment. However, the amount of **Sos** co-immunoprecipitated with Grb2 was apparently not affected by Dexa treatment. These results indicate that Grb2 and **Sos** constitutively associate with each other in U937 cells. Furthermore, the level of phosphorylation on tyrosine (204) essential for MAP kinase activation was decreased after Dexa treatment. Taken together with all these findings, it is suggested that c-Src directly phosphorylates Shc on tyrosine residues, which in turn binds to Grb2 constitutively associated with **Sos** to form a Shc-Grb2-**Sos** complex, and that the complex formation is coupled with MAP kinase activation mediated by Ras activation in U937 cells.

3/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09256095 97158232 PMID: 9004504
Structural and functional organization of the Yersinia pestis bacteriocin pesticin gene cluster.
Rakin A; Boolgakowa E; Heesemann J
Max von Pettenkofer Institut fur Hygiene und Medizinische Mikrobiologie, Universitat Munchen, Germany.
Microbiology (Reading, England) (ENGLAND) Dec **1996**, 142 (Pt 12) p3415-24, ISSN 1350-0872 Journal Code: 9430468
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The primary structure of a 2671 bp DNA fragment between the pla gene (encoding plasminogen activator) and the origin of replication of the wild-type Yersinia pestis plasmid pYP358 was determined. Two ORFs of 1074 and 426 bp with opposite transcription polarities were identified on both strands. They encode a 357 aa pesticin activity protein (Pst) and a 141 aa pesticin immunity polypeptide (Pim). A GC-rich palindromic structure located between pst and pim can form a hairpin loop and serve as rho-independent transcription terminator sequences for both genes. The site for the interaction with the LexA repressor of the **SOS** system was found in another palindromic structure preceding the pst structural gene. A deduced 39.9 kDa Pst polypeptide is devoid of a signal peptide, indicating a Sec-independent mode of export. Pst carries a pentapeptide typical of TonB-dependent colicins (TonB box) that is necessary for the interaction with the yersiniabactin/pesticin receptor and for active energy-dependent transport through the outer membrane. The substitution of the last five C-terminal amino acids did not significantly influence the bactericidal activity of the truncated pesticin. The pesticin lost its ability to kill sensitive bacteria and to bind to a pesticin receptor after deletion of the last 57 C-terminal amino acids. A deduced 16 kDa Pim protein has an N-terminal hydrophobic amino acid stretch with features typical of prokaryotic signal peptides. Pim is a slightly hydrophilic protein with a basic pI. The immunity protein was localized in the periplasmic space and in the outer-membrane fraction after its overexpression under the polymerase T7 promoter. Several other ORFs were identified on the sequenced 2671 bp fragment, but none of them seemed to encode a typical lysis peptide, which is necessary for the release of the pesticin. In the promoter region and in the regions preceding and following the pst operon, the DNA sequence has high (> 70%) identity with other colicin genes. The DNA sequence located 284 bp upstream of the pim gene showed more than 90% similarity to **antisense** RNA I of the ColE1 replicon. This defined the location of the pYP358 origin of ColE1-type replication.

3/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09016785 96371012 PMID: 8774847
Catalytic activities of hammerhead **ribozymes** with a triterpenoid linker instead of stem/loop II.
Sugiyama H; Hatano K; Saito I; Amontov S; Taira K
Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Japan.
FEBS letters (NETHERLANDS) Sep 2 **1996**, 392 (3) p215-9, ISSN 0014-5793 Journal Code: 0155157
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A minizyme is a hammerhead **ribozyme** with short oligonucleotide linkers instead of stem/loop II. In a previous study we demonstrated that a minizyme with high-level activity forms a dimeric structure with a common stem II (Amontov and Taira, J. Am. Chem. **Soc**., 118 (1996) 1624-1628). As a continuation of this study, we recently examined whether a short oligonucleotide linker at stem/loop II could be replaced by a triterpenoid linker and whether the resulting minizymes with bulky hydrophobic groups would form dimeric structures. In contrast to the minizyme with high-level activity that we characterized in the previous study, minizymes that contained a triterpenoid group had low cleavage activities. The nature of the dependence of the activity on the concentration of **ribozyme** revealed that these minizymes with a triterpenoid group do not form dimeric structures. Thus, the low activities of these structures can be attributed to their failure to form dimers.

3/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08877382 96228043 PMID: 8647298
Dependence on Mg2+ ions of the activities of dimeric hammerhead minizymes.
Amontov S; Nishikawa S; Taira K
National Institute of Bioscience and Human Technology, Tsukuba Science City, Japan.
FEBS letters (NETHERLANDS) May 20 **1996**, 386 (2-3) p99-102, ISSN 0014-5793 Journal Code: 0155157
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A minizyme is a hammerhead **ribozyme** with short oligonucleotide linkers instead of stem/loop II. In a previous study we demonstrated that a minizyme with high-level activity forms a dimeric structure with a common stem II [Amontov and Taira (1996) J. Am. Chem. **Soc**. 118, 1624-1628]. We now demonstrate that the stability of the dimeric structure is influenced by Mg2+ ions. We found that the dependence on Mg2+ ions of the activity of homodimeric minizyme (a dimer with two identical binding sites) has composite biphasic characteristics. When the concentration of Mg2+ ions reached a specific critical level, the dependence on the concentration of Mg2+ ions lost its tendency to reach a plateau. In the case of the heterodimeric minizyme (a dimer with two different binding sites), we investigated the kinetic behavior of two different forms of the dimer, namely, free dimer and the complex of the dimer with an uncleavable substrate. The kinetic behavior of the free heterodimer was very similar to that of the homodimeric minizyme. In contrast, in the presence of the uncleavable substrate at a concentration as high as that of the minizyme, the curve for the dependence on Mg2+ ions showed normal saturation kinetics. While, at low concentrations of Mg2+ ions, the activity of the heterodimers was much higher when the dimeric structure was stabilized by the presence of the uncleavable substrate, at high concentrations of Mg2+ ions, this difference in activity became less and less significant. Thus, high concentrations of Mg2+ ions were able to stabilize the dimeric minizymes in the absence of the uncleavable substrate.

3/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08543342 95302484 PMID: 7783193
Mechanism of post-segregational killing by hok-homologue pnd of plasmid R483: two translational control elements in the pnd mRNA.
Nielsen A K; Gerdes K
Department of Molecular Biology, Odense University, Denmark.
Journal of molecular biology (ENGLAND) Jun 2 **1995**, 249 (2) p270-82, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The pnd system of plasmid R483 mediates plasmid stabilization by killing of plasmid-free cells. The pnd mRNA is very stable and can be translated into PndA protein, a cell toxin which kills the cells from within by damaging the cell membrane. Translation of the pnd mRNA is inhibited by the **PndB antisense**, a small labile RNA of 63 nt. The rapid decay of the **PndB** antidote leads to onset of PndA synthesis in plasmid-free segregants or after addition of rifampicin. Surprisingly however, the

5/3,AB/2 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10886989 BIOSIS NO.: 199799508134
Antisense oligodeoxynucleotides to opioid mu and delta receptors reduced morphine dependence in mice: Role of delta-2 opioid receptors.
AUTHOR: Sanchez-Blazquez Pilar(a); Garcia-Espana Antonio; Garzon Javier
AUTHOR ADDRESS: (a)Neurofarmacol., Inst. Cajal, C.S.I.C., C/Doctor Arce, 37, 28002 Madrid**Spain
JOURNAL: Journal of Pharmacology and Experimental Therapeutics 280 (3):p 1423-1431 **1997**
ISSN: 0022-3565
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Repeated intracerebroventricular injections of **antisense** oligodeoxynucleotides (ODNs) were used to selectively restrict the expression of cloned mu and delta opioid receptors (OR) in the mouse brain. Reduction of mu and delta OR-like immunoreactivity was observed in brain structures of experimental mice. A random-sequence ODN used as a control showed no effect. ODNs to OR decreased radiolabeling of neural structures after intracerebroventricular injection of 125I-immunoglobulins G directed to mu or delta OR. The potencies of opioids binding the mu OR, (D-Ala-2,N-MePhe-4,Gly-ol-5)enkephalin and morphine were significantly attenuated in mice injected with ODNs to this receptor, an effect not seen for the delta OR-binding agonists, (D-Pen-2,5)enkephalin and (D-Ala-2)deltorphin II. In morphinedependent mice, ODNS to mu OR reduced the incidence of naloxone-precipitated withdrawal jumping, body weight loss and diarrhea. The ODN directed to nucleotides 7-26 of the delta OR mRNA selectively impaired antinociception induced by (D-Ala-2)deltorphin II (delta-2), but not that of (D-Pen-2,5)enkephalin (delta-1) or morphine. It also diminished the incidence of withdrawal signs precipitated by naloxone in morphine-dependent mice. Thus, the cloned mu OR mediates morphine-evoked antinociception as well as physical dependence. The involvement of delta-2 OR in the development and/or expression of morphine dependence is suggested.

1997

5/3,AB/3 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10850231 BIOSIS NO.: 199799471376
Cell death in bacterial populations: Caused or programmed? Accepted or deliberate?
AUTHOR: Boutibonnes Philippe
AUTHOR ADDRESS: Lab. Genet. Microbienne, Univ. Caen, 14032 Caen**France
JOURNAL: M-S (Medecine Sciences) 13 (1):p73-80 **1997**
ISSN: 0767-0974
RECORD TYPE: Abstract
LANGUAGE: French; Non-English
SUMMARY LANGUAGE: French; English

ABSTRACT: In bacterial cells, plasmids are stabilized by numerous different mechanisms. Here we describe three different ways which mediate plasmid maintenance by selectively killing plasmid free cells. In the first system, plasmid genes encode a stable toxic protein (or the stable corresponding mRNA) and an unstable **antidote** (or a small unstable

antisens RNA which inhibits the translation of the messenger). The expression of the lethality is regulated post-translationally (e.g. ccd system from plasmid F in Escherichia coli) or post-transcriptionally (e.g. hok system from plasmid R1 in E. coli). In the second system (e.g. rm genes of Pseudomonas aeruginosa R7) plasmid genes encode a stable type II restriction enzyme and the unstable cognate methylase which offers protection from endonucleolytic attack by the restriction enzyme. The third system is composed of a complex operon (e.g. MccB17 system in E. coli) whose genes encode the synthesis and maturation of a cytotoxic protein and for a polypeptide which confers resistance or "immunity" to the killer cell by an unknown mechanism. The plasmid-free cells are killed by the cytotoxic protein excreted by bacteria carrying the plasmid. The set of two or more genes carried by a plasmid responsible for the lethal consequences of plasmid loss can be viewed as an "addiction module" or a "selfish symbiot" according to Yarmolinsky and Naito, respectively; the loss of these genomic units lead the cells to an unavoidable death.

1997

5/3,AB/4 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10368585 BIOSIS NO.: 199698823503
Functional analysis of the Enterococcus faecalis plasmid pAD1-encoded stability determinant par.
AUTHOR: Weaver Keith E(a); Jensen Kristi D; Colwell Any; Sriram Sai Leela
AUTHOR ADDRESS: (a)Dep. Microbiol., Sch. Med., Univ. South Dakota, Vermillion, SD 57069**USA
JOURNAL: Molecular Microbiology 20 (1):p53-63 **1996**
ISSN: 0950-382X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The molecular organization and functional characteristics of the pAD1 replicon-encoded par stability determinant were examined. par encodes two convergently transcribed RNAs of apprxeq 210 and 65 nucleotides designated RNA I and RNA II, respectively. The sequence of RNA II is largely complementary to RNA I, suggesting that RNA II could regulate RNA I function as an **antisense** RNA. Results of functional studies are consistent with a role for par as a post-segregational killing system, the first to be identified in Gram-positive bacteria, with RNA I encoding the toxin and RNA II the **antidote**. These results include: (i) destabilization of par-containing replicons in the presence of a second complete par or the RNA II coding sequence in the same cell; (ii) par-dependent stabilization of a highly unstable vector at the expense of host-cell growth rate; and (iii) protection of cells from the toxic effects of overexpression of RNA I by RNA II supplied in trans.

1996

5/3,AB/5 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09558727 BIOSIS NO.: 199598013645
Synthesis of oligonucleotides containing 1-(beta-D-3'-deoxy-threo-pentofuranosyl)pyrimidines and their resistance to the action of snake venom phosphodiesterase.

AUTHOR: Oretskaya T S; Ibragim Kh K Kh; Volkov E M; Romanova E A; Tashlitskii V N; Shabarova Z A
AUTHOR ADDRESS: Chem. Fac., M.V. Lomonosov Mosc. State Univ., Vorob'evy gory, 119899 Moscow**Russia
JOURNAL: Bioorganicheskaya Khimiya 20 (8-9):p967-974 **1994**
ISSN: 0132-3423
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Russian; Non-English
SUMMARY LANGUAGE: Russian; English

ABSTRACT: Effective methods of multiple incorporations of nucleotides with the inverted configuration of C2' hydroxyl group have been developed. The presence of tU and tC as 3'-terminal oligonucleotide modifications is shown to increase their resistance to snake venom phosphodiesterase. The obtained compounds are promising for the use in **antisense** biotechnology.

1994

5/3,AB/6 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08629513 BIOSIS NO.: 199345047588
Reversal of teratogenic effects of retinoic acid by **antisense** RAR-beta-2 deoxyoligonucleotides in mouse embryo limb bud cells in vitro.
AUTHOR: Jiang H; Soprano D R; Soprano K J; Kochhar D M
AUTHOR ADDRESS: Dep. Anatomy Dev. Biology, Thomas Jefferson Univ., Philadelphia, PA**USA
JOURNAL: Teratology 47 (5):p423-424 **1993**
CONFERENCE/MEETING: Thirty-third Annual Meeting of the Teratology Society Tucson, Arizona, USA June 28-July 1, 1993
ISSN: 0040-3709
RECORD TYPE: Citation
LANGUAGE: English
1993

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Set     Items   Description
S1         97   (CCDA OR KIS OR PEMI OR PARD OR PHD OR HIGA OR CHPAI OR CH-
              PBI OR KICA OR SOC OR SOS OR SRNC OR FLMB OR PNDB OR SOF OR K-
              ORA OR KORB OR KORC OR KORD OR KORE OR KORF) AND (ANTISENSE OR
               RIBOZYM?)
S2         46   S1 AND PY<1999
S3         29   RD (unique items)
S4          7   ANTIDOT? AND (ANTISENS? OR RIBOZYM?) AND PY<1999
S5          6   RD (unique items)
? s addiction and (antisens? or ribozym?) and py<1999
           19888  ADDICTION
           36929  ANTISENS?
            6386  RIBOZYM?
         21931987  PY<1999
      S6       9  ADDICTION AND (ANTISENS? OR RIBOZYM?) AND PY<1999
? rd
...completed examining records
      S7       7  RD (unique items)
? s s7 not s5
               7  S7
               6  S5
      S8       6  S7 NOT S5
? t s8/3,ab/all

 8/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

10155582   99163420   PMID: 10068276
  Disorders in cell circuitry associated with multistage carcinogenesis:
exploitable targets for cancer prevention and therapy.
  Weinstein I B; Begemann M; Zhou P; Han E K; Sgambato A; Doki Y; Arber N;
Ciaparrone M; Yamamoto H
  Herbert Irving Comprehensive Cancer Center, Columbia University College
of Physicians and Surgeons, New York, NY 10032, USA.
  Clinical cancer research : an official journal of the American
Association for Cancer Research (UNITED STATES)  Dec 1997, 3 (12
Pt 2) p2696-702, ISSN 1078-0432  Journal Code: 9502500
  Contract/Grant No.: CA26056; CA; NCI; CA63467; CA; NCI
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  The development of a malignant tumor involves the progressive acquisition
of mutations and epigenetic abnormalities in multiple genes that have
highly diverse functions. Some of these genes code for pathways of signal
transduction that mediate the action of growth factors. The enzyme protein
kinase C plays an important role in these events and in the process of
tumor promotion. Therefore, we examined the effects of three inhibitors of
protein kinase C, CGP 41251, RO 31-8220, and calphostin C, on human
glioblastoma cells. These compounds inhibited growth and induced apoptosis;
these activities were associated with a decrease in the level of CDC2 and
cyclin B1/CDC2-associated kinase activity. This may explain why the treated
cells accumulated in G2-M. In a separate series of studies, we examined
abnormalities in cell cycle control genes in human cancer. We have found
that cyclin D1 is frequently overexpressed in a variety of human cancers.
Mechanistic studies indicate that cyclin D1 can play a critical role in
carcinogenesis because: overexpression enhances cell transformation and
tumorigenesis; introduction of an antisense cyclin D1 cDNA into
either human esophageal or colon cancer cells reverts their malignant
phenotype; and overexpression of cyclin D1 can enhance the amplification of
other genes. The latter finding suggests that cyclin D1 can enhance genomic
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instability and, thereby, the process of tumor progression. Therefore, inhibitors of the function of cyclin D1 may be useful in both cancer chemoprevention and therapy. We obtained evidence for the existence of homeostatic feedback loops between cyclins D1 or E and the cell cycle inhibitory protein p27Kip1. On the basis of these and other findings, we hypothesize that, because of their disordered circuitry, cancer cells suffer from "gene **addiction**" and "gene hypersensitivity," disorders that might be exploited in both cancer prevention and therapy.

8/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09505207 97406809 PMID: 9260191
Biocatalysts in detoxication of drugs of abuse.
Cashman J R
Seattle Biomedical Research Institute, WA 98109, USA.
NIDA research monograph (UNITED STATES) **1997**, 173 p225-58,
ISSN 1046-9516 Journal Code: 8811762
Contract/Grant No.: DA08531; DA; NIDA; ES 06973; ES; NIEHS; GM 36426; GM; NIGMS
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Currently there is a significant amount of information about the way biocatalysts from animals detoxicate and bioactive drugs of abuse. In some cases, biotransformation data concerning drugs of abuse obtained from animal systems are analogous to the human situation, but in many cases the data are not. Clearly, significant work needs to be done with human biocatalysts to define a role in the biotransformation of drugs of abuse and to relate the work that has already been done in animals. New metabolic pathways will likely be discovered that may link drug metabolism to **addiction** liability or drug susceptibility in humans. New design and selection technologies are providing the basis to allow the discovery of new biocatalysts that may be useful in the detoxication of drugs of abuse in humans. Fundamentally new approaches using biocatalysts including rationally engineered enzymes, catalytic antibodies, catalytic antibody fragments, **ribozymes** , oligonucleotides, and other biomacromolecules may provide basic information that may later support the rational design of biocatalysts, which may in turn provide the basis for designing detoxication catalysts for drugs of abuse.

8/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08985467 96363008 PMID: 8750825
Increased expression of synapsin I mRNA in defined areas of the rat central nervous system following chronic morphine treatment.
Matus-Leibovitch N; Ezra-Macabee V; Saya D; Attali B; Avidor-Reiss T; Barg J; Vogel Z
Department of Neurobiology, Weizmann Institute of Science, Israel.
Brain research. Molecular brain research (NETHERLANDS) Dec 28 **1995**, 34 (2) p221-30, ISSN 0169-328X Journal Code: 8908640
Contract/Grant No.: DA-6265; DA; NIDA
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Chronic opiate administration leads to a selective regulation of several cellular proteins and mRNAs. This phenomenon has been viewed as a

compensatory mechanism to the opiate signaling leading to the development of opiate **addiction** . In this study, in situ hybridization histochemistry experiments were employed to investigate the effect of chronic morphine treatment on synapsin I gene expression. We show here for the first time that prolonged morphine exposure causes a selective increase in the mRNA levels of synapsin I in several brain regions which are considered to be important for opiate action. Quantitative analysis of the signals, obtained by hybridization of digoxigenin-labeled **antisense** RNA probe, revealed a 5.8- and 7-fold increase of synapsin I mRNA levels in the locus coeruleus and the amygdala of morphine-treated rats, respectively, as compared with control untreated rats. Increased expression of synapsin I mRNA was also observed in the spinal cord of morphine-treated rats (by 3.8-fold). Since opiates were shown to attenuate neurotransmitter release and reduce synapsin I phosphorylation, it is suggested that the increase in synapsin I levels would lead to the requirement of higher amounts of opiate agonists to obtain the opiate physiological effects. These results suggest that the increases in mRNA levels of synapsin I in these specific areas can be part of the molecular mechanism(s) underlying opiate tolerance and withdrawal.

8/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08797832 96135082 PMID: 8558448
Regulation of CREB expression: in vivo evidence for a functional role in morphine action in the nucleus accumbens.
Widnell K L; Self D W; Lane S B; Russell D S; Vaidya V A; Miserendino M J ; Rubin C S; Duman R S; Nestler E J
Department of Psychiatry, Yale University School of Medicine, New Haven, Connecticut, USA.
Journal of pharmacology and experimental therapeutics (UNITED STATES) Jan **1996**, 276 (1) p306-15, ISSN 0022-3565 Journal Code: 0376362
Contract/Grant No.: DA00203; DA; NIDA; DA07359; DA; NIDA; DA08227; DA; NIDA; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Previous work has shown that chronic opiate administration regulates protein components of the cAMP signaling pathway, specifically in the nucleus accumbens (NAc), a brain region implicated in the reinforcing properties of opiates, and that such adaptations may contribute to changes in reinforcement mechanisms that characterize opiate **addiction**. In the present study, we examined a possible role for the transcription factor cAMP response element-binding protein (CREB) in mediating these long-term effects of opiates in the NAc. Chronic, but not acute, morphine administration was found to decrease levels of CREB immunoreactivity in the NAc, an effect not seen in other brain regions studied. The functional significance of this CREB down-regulation was then investigated by the use of an anti-sense oligonucleotide strategy that produces a specific and sustained decrease in CREB levels in the NAc, without detectable toxicity. It was found that the **antisense** oligonucleotide-induced reduction in CREB levels mimicked the effect of morphine on certain, but not all, cAMP pathway proteins in this brain region, whereas a large number of other signal transduction proteins tested were unaffected by this treatment. Our results support a role for CREB in autoregulation of the cAMP pathway in the nervous system, as well as in mediating some of the effects of morphine on this signaling pathway in the NAc.

8/3,AB/5 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11131615 BIOSIS NO.: 199799752760
RG-beta-1: A psychostimulant-regulated gene essential for establishing cocaine sensitization.
AUTHOR: Wang Xiao-Bing; Funada Masahiko; Imai Yasuo; Revay Randal S; Ujike Hiroshi; Vandenbergh David J; Uhl George R(a)
AUTHOR ADDRESS: (a)Molecular Neurobiol., Box 5180, Baltimore, MD 21224**USA
JOURNAL: Journal of Neuroscience 17 (15):p5993-6000 **1997**
ISSN: 0270-6474
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Repeated doses of cocaine or amphetamine lead to long-lasting behavioral manifestations that include enhanced responses termed sensitization. Although biochemical mechanisms that underlie these manifestations currently remain largely unknown, new protein synthesis has been implicated in several of these neuroadaptive processes. To seek candidate biochemical mechanisms for these drug-induced neuroplastic behavioral responses, we have used an approach termed subtracted differential display (SDD) to identify genes whose expression is regulated by these psychostimulants. rG-beta-1 is one of the SDD products that encodes a rat G-protein beta subunit. rG-beta-1 expression is upregulated by cocaine or amphetamine treatments in neurons of the nucleus accumbens shell region, a major center for psychostimulant effects in locomotor control and behavioral reward. **Antisense** oligonucleotide treatments that attenuate rG-beta-1 expression in regions including the nucleus accumbens abolish the development of behavioral sensitization when they are administrated during the repeated cocaine exposures that establish sensitization. These treatments fail to alter acute behavioral responses to cocaine, and they do not block the expression of cocaine sensitization when it is established before oligonucleotide administrations. Full, regulated rG-beta-1 expression is a biochemical component essential to the establishment of a key consequence of repeated cocaine administrations, sensitization.

1997

8/3,AB/6 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10649560 BIOSIS NO.: 199699270705
Effects of acute and chronic infusions of CREB **antisense** oligonucleotides in the nucleus accumbens on cocaine self-administration.
AUTHOR: Self D W; Spencer J J; Nestler E J
AUTHOR ADDRESS: Div. Mol. Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06513**USA
JOURNAL: Society for Neuroscience Abstracts 22 (1-3):p1882 **1996**
CONFERENCE/MEETING: 26th Annual Meeting of the Society for Neuroscience Washington, D.C., USA November 16-21, 1996
ISSN: 0190-5295
RECORD TYPE: Citation
LANGUAGE: English
1996

on Ras, etc.); alteration of essential covalent modifications (i.e., farnesylation of Ras which is essential for its association with the plasma membrane); and various forms of gene therapy to introduce genes (i.e., addition of wild-type p53) or to reduce activity of genes essential for growth (i.e., dominant negative receptor mutants). 2) Interfere with protein-protein or DNA-protein interactions that are needed for the activity of oncogenes and/or growth factors or the transcription factors essential for cell growth. This approach has been demonstrated to work in vitro to interfere with SH2-tyrosine phosphate interactions (i.e., Grb-2 and EGF receptor) and Ras-Raf interactions using specific peptides (J. Downward), but to be useful therapeutically it must be possible to introduce stable low-molecular-weight drugs into cells to affect these interactions.(ABSTRACT TRUNCATED AT 400 WORDS)

3/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07994652 94137806 PMID: 8305516
2',5'-Oligoadenylate:**antisense** chimeras--synthesis and properties.
Lesiak K; Khamnei S; Torrence P F
Section on Biomedical Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892.
Bioconjugate chemistry (UNITED STATES) Nov-Dec **1993**, 4 (6) p467-72, ISSN 1043-1802 Journal Code: 9010319
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have synthesized a novel bioconjugate which joins an **antisense** oligonucleotide to a unique and potent inhibitor of translation,pn5'A2'(p5'A2')mp5'A(2-5A). Two residues of 4-hydroxybutyl phosphate were employed as linkers to attach the 2',5'-oligoadenylate moiety through its 2'-terminus to the 5'-terminus of the chosen **antisense** sequence, (dT)20. The syntheses were carried on a solid support according to the phosphite triester method of DNA synthesis (Letsinger, R.L., Lunsford, W.B. (1976) J. Am. Chem. **Soc**. 98, 3655-3661; Beaucage, S.L., and Caruthers, M.H. (1981) Tetrahedron Lett. 22, 1859-1862). The generated 2-5A **antisense** chimeras retained both the ability of the 2-5A molecule to activate the 2-5A-dependent RNase as well as the ability of the oligo(dT) moiety to hybridize to the complementary poly(A). Moreover, the chimera, when annealed to its target nucleic acid sequence, was still effectively bound to the 2-5A-dependent nuclease. The methodology described represents a new approach to the selective modulation of mRNA expression.

3/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07919394 94049025 PMID: 8231732
Structure of the human gene for the neural phosphoprotein B-50 (GAP-43).
Nielander H B; De Groen P C; Eggen B J; Schrama L H; Gispen W H; Schotman P
Division of Molecular Neurobiology, Rudolf Magnus Institute, Utrecht University, The Netherlands.
Brain research. Molecular brain research (NETHERLANDS) Sep **1993**, 19 (4) p293-302, ISSN 0169-328X Journal Code: 8908640
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed
The genomic DNA encoding the exons for the human neural phosphoprotein B-50 (GAP-43) was isolated using rat-based cDNA probes and oligonucleotides. Exons 2 and 3 were isolated from a genomic library, exon 1 was amplified by PCR on total genomic DNA. The gene consists of 3 exons and 2 large introns. The first exon encodes the N-terminal 10 amino acids of B-50 involved in membrane association of the protein. Exon 2 encodes the main part of the protein with the sites for protein kinase C-mediated phosphorylation and calmodulin binding, and includes a 10 amino acid residue insert not found in rodents. Exon 3 encodes the last 29 amino acid residues. The reported sequence extends the known cDNA structure to both the 5' and 3' ends. The 358 bp region upstream of the translational initiation codon, containing the main transcription starts, is purine-rich and does not include TATA or GC boxes. At the 3' end potential polyadenylation signals were found 510 bp and 584 bp downstream of the stopcodon in exon 3. The 5' end of the mRNA is heterogeneous in length, with primer extension products corresponding to a 5' untranslated region of 159 and 343 bases. Northern hybridizations, however, indicate that the majority of B-50 mRNA has a shorter 5' untranslated region, as was reported for the rat (Schrama et al., **Soc**. Neurosci. Abstr., 18 (1992) 333.4).
The structural organization of the human gene is similar to that described for the rat (Grabczyk et al., Eur. J. Neurosci. 2 (1990) 822-827), and both translated and untranslated regions show a high degree of sequence homology to the rat gene.

3/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07611712 93121964 PMID: 8419145
The regulation by growth hormone of lipoprotein lipase gene expression is mediated by c-fos protooncogene.
Barcellini-Couget S; Pradines-Figueres A; Roux P; Dani C; Ailhaud G
Centre de Biochimie (UMR 134 CNRS), Universite de Nice-Sophia Antipolis, UFR Sciences, France.
Endocrinology (UNITED STATES) Jan **1993**, 132 (1) p53-60, ISSN 0013-7227 Journal Code: 0375040
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
GH has been previously shown in Ob1771 adipose cells to activate transiently the expression of c-fos gene by a protein kinase-C-dependent pathway and to modulate, at last in part by a protein kinase-C-dependent pathway, the expression of the lipoprotein lipase (LPL) gene. In Ob1771 cells exposed to GH, under conditions where protein synthesis is inhibited by cycloheximide, the modulation of LPL gene expression is prevented, suggesting that synthesis of trans-acting factor(s) is required to modulate LPL gene expression. The present results indicate the involvement of c-Fos protein in this modulation; this involvement is supported by various lines of evidence: 1) upon GH stimulation, the increase in c-fos mRNA content is followed by the emergence of c-Fos protein within the nucleus, and this emergence precedes the increase in LPL mRNA content; 2) in GH-treated Ob1771 cells, exposure to **antisense sof** oligonucleotides abolishes the synthesis of c-Fos protein; and 3) at the same time, the increase in LPL mRNA content and LPL activity does not occur, whereas sense fos oligonucleotides show no effect. It is concluded that c-Fos protein plays an intermediary role in the modulation of LPL gene expression by GH.

3/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07588051 93106972 PMID: 8416908
Complete nucleotide sequence of a linear plasmid from Streptomyces clavuligerus and characterization of its RNA transcripts.
Wu X; Roy K L
Department of Microbiology, University of Alberta, Edmonton, Canada.
Journal of bacteriology (UNITED STATES) Jan **1993**, 175 (1) p37-52, ISSN 0021-9193 Journal Code: 2985120R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The complete nucleotide sequence of a small linear plasmid (pSCL1) from Streptomyces clavuligerus has been determined. This plasmid is 11,696 bp in length, has a 72% G+C content, and has approximately 900-bp inverted terminal repeat sequences. A comparison of the inverted terminal repeats of pSCL1 with those of a linear plasmid from S. rochei shows that the two terminal sequences have a high degree of similarity (approximately 70%). Several small inverted repeats found in the long terminal sequences of both plasmids are also conserved. An analysis of the sequence and codon preferences indicates that pSCL1 has seven or eight highly probable protein-coding open reading frames (ORFs). However, only two RNA species encoded by pSCL1 were detected in S. clavuligerus grown in liquid culture. The larger of these transcripts (900 nucleotides) corresponds to an ORF and is likely to be an mRNA for a protein similar to the **KorA** protein of pIJ101. The smaller transcript (460 nucleotides) does not correspond to any ORF; however, its 5' end is complementary to the 5' end of a predicted mRNA, suggesting that it may function as an **antisense** RNA. The larger of the two RNA species was present at a high level during the early stage of growth in liquid medium, and then its apparent rate of transcription decreased and remained at a lower level through the later stages; the level of the smaller RNA species remained relatively constant through all stages of growth.

3/3,AB/19 (Item 19 from file: 155)

07281791 92212879 PMID: 1372984
What is the optimum size for the genetic alphabet?
Szathmary E
Laboratory of Mathematical Biology, National Institute for Medical Research, Mill Hill, London, United Kingdom.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Apr 1 **1992**, 89 (7) p2614-8, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
An important question in biology is why the genetic alphabet is made of just two base pairs (G.C and A.T). This is particularly interesting because of the recent demonstration [Piccirilli, J. A., Krauch, T., Moroney, S. E. & Benner, S. A. (1990) Nature (London) 343, 33-37] that the alphabet can in principle be larger. It is possible to explain the size of the present genetic alphabet as a frozen character state that was an evolutionary optimum in an RNA world when nucleic acids functioned both for storing genetic information and for expressing information as enzymatically active RNA molecules--i.e., **ribozymes** . A previous model [Szathmary, E. (1991) Proc. R. **Soc** . London Ser. B 245, 91-99] has described the principle of this approach. The present paper confirms and extends these results by showing explicitly the ways in which copying fidelity and metabolic efficiency change with the size of the genetic alphabet.

3/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07178297 92114772 PMID: 1722558
The rifampicin-inducible genes srnB from F and pnd from R483 are regulated by **antisense** RNAs and mediate plasmid maintenance by killing of plasmid-free segregants.
Nielsen A K; Thorsted P; Thisted T; Wagner E G; Gerdes K
Department of Molecular Biology, Odense University, Denmark.
Molecular microbiology (ENGLAND) Aug **1991**, 5 (8) p1961-73, ISSN 0950-382X Journal Code: 8712028
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The gene systems srnB of plasmid F and pnd of plasmid R483 were discovered because of their induction by rifampicin. Induction caused membrane damage, RNase I influx, degradation of stable RNA and, consequently, cell killing. We show here that the srnB and pnd systems mediate efficient stabilization of a mini-R1 test-plasmid. We also show that the killer genes srnB' and pndA are regulated by **antisense** RNAs, and that the **srnC**- and **pndB**-encoded **antisense** RNAs, denoted **SrnC**- and **PndB** -RNAs, are unstable molecules of approximately 60 nucleotides. The srnB and pndA mRNAs were found to be very stable. The differential decay rates of the inhibitory **antisense** RNAs and the killer-gene-encoding mRNAs explain the induction of these gene systems by rifampicin. Furthermore, the observed plasmid-stabilization phenotype associated with the srnB and pnd systems is a consequence of this differential RNA decay: the newborn plasmid-free cells inherit the stable mRNAs, which, after decay of the unstable **antisense** RNAs, are translated into killer proteins, thus leading to selective killing of the plasmid-free segregants. Thus our observations lead us to conclude that the F srnB and R483 pnd systems are phenotypically indistinguishable from the R1 hok/sok system, despite a 50% dissimilarity at the level of DNA sequence.

3/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06992223 91329297 PMID: 2101633
The hok killer gene family in gram-negative bacteria.
Gerdes K; Poulsen L K; Thisted T; Nielsen A K; Martinussen J; Andreasen P H
Department of Molecular Biology, Odense University, Denmark.
New biologist (UNITED STATES) Nov **1990**, 2 (11) p946-56, ISSN 1043-4674 Journal Code: 9000976
Document type: Journal Article; Review; Review, Academic
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The seven members of the hok killer gene family in Gram-negative bacteria are described here. The members of this gene family have been sequenced and include hok/sok from plasmid R1, flm and srnB from plasmid F, pnd from plasmids R483 and R16, and gef and relF, which are located on the Escherichia coli chromosome. The killer proteins encoded by these loci are highly toxic polypeptides of 50 to 52 amino acids. The proteins kill the cells from the inside by interfering with a vital function in the cell membrane. On the basis of their relatedness, the killer proteins and their corresponding loci are divided into four subfamilies. The members of one

subfamily, hok/sok and flm, mediate plasmid maintenance by killing plasmid-free cells. The pnd and srnB subfamilies were discovered through their abilities to cause membrane damage and degradation of stable RNA. gef and relF, which constitute the chromosomal subfamily, were found because of their sequence similarity at the DNA and protein levels with other members of the hok gene family. However, no function has been described for the proteins belonging to this subfamily. Although the four subfamilies are distantly related in terms of DNA and protein sequence similarity, the overall genetic organization of the different loci has been well conserved during evolution. The expression of all of the members of the hok gene family is regulated post-transcriptionally. Thus, the expression of the hok and flm genes is regulated by small **antisense** RNAs that inhibit the translation of the stable hok and flm mRNAs. On the basis of structural and functional similarities, we suggest that each of the related plasmid-encoded killer genes is regulated by **antisense** RNAs. The conservation of this widespread gene family in Gram-negative bacteria suggests that the genes are important to the genomes that carry them.

3/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06855882 91156293 PMID: 1847998
mos-induced inhibition of glucocorticoid receptor function is mediated by Fos.
Touray M; Ryan F; Saurer S; Martin F; Jaggi R
Department of Clinical and Experimental Cancer Research, University of Bern, Switzerland.
Oncogene (ENGLAND) Feb **1991**, 6 (2) p211-7, ISSN 0950-9232
Journal Code: 8711562
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Activation of glucocorticoid hormone-dependent transcription involves the binding of the glucocorticoid hormone to its receptor followed by a specific interaction of the hormone/receptor complex with glucocorticoid responsive elements in the promoter region of hormone-inducible genes. In stably transfected NIH3T3 cells expressing the oncogene product of v-mos or fos, the expression from two glucocorticoid responsive promoters, MMTV LTR and metallothionein IIA (MtIIA), was shown to be impaired and was only transient. Cadmium-dependent MtIIA gene expression was not affected by the expression of v-mos in the cells. In transiently transfected NIH3T3 cells constitutive fos expression also inhibited glucocorticoid hormone-induced expression from the MMTV LTR. However, co-expression of **antisense** fos (here referred to as **sof**) inhibited the down-regulatory effect of Fos on glucocorticoid induced gene expression. v-mos expression in NIH3T3 cells induces fos mRNA and functional fos product (Fos) as reflected by its ability to induce expression of a transiently transfected AP-1 dependent reporter plasmid. We show that **sof** expression inhibits the down-regulatory effect of mos on expression of a transiently transfected pMMTV LTR-CAT. Our findings, thus, strongly suggest that the inhibition of glucocorticoid receptor function in cells expressing the v-mos oncogene is mediated by Fos.

3/3,AB/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06227708 89313301 PMID: 2473377
Transcription of the sulA-ompA region of Escherichia coli during the **SOS** response and the role of an **antisense** RNA molecule.

Cole S T; Honore N
Laboratoire de Genetique Moleculaire Bacterienne, Institut Pasteur, Paris, France.
Molecular microbiology (ENGLAND) Jun **1989**, 3 (6) p715-22, ISSN 0950-382X Journal Code: 8712028
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The transcriptional pattern of the 22 min region of the Escherichia coli chromosome containing the linked sulA and ompA genes, which encode an **SOS** -inducible inhibitor of cell division and a constitutively expressed, major outer membrane protein, respectively, has been re-examined. During normal growth, the sulA gene was repressed whereas the ompA gene produced a stable 1250 nucleotide transcript. Counter-transcription of sulA occurred from a promoter situated in the sulA-ompA intergenic region and the product of this transcriptional circuit, named isf, is a 353 nucleotide untranslated RNA. Since the isf RNA is complementary to the 3'-end of the sulA transcript, it could modulate sulA function by serving as an anti-messenger. On induction of the **SOS** -response, massive transcription of sulA took place, resulting in the 'silencing' of the isf gene, production of an abundant approximately 615 nucleotide sulA mRNA and a novel hybrid transcript of approximately 2100 nucleotides encoding both the SulA and OmpA proteins. Production of the latter RNA species, caused by transcription reading through the sulA terminator, the intergenic region and the coding sequences, was accompanied by a decrease in the abundance of the ompA mRNA as a result of promoter occlusion. However, the amount of OmpA protein produced was only slightly reduced.

3/3,AB/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05935337 89006267 PMID: 3049248
Nucleotide sequence and transcriptional analysis of a third function (Flm) involved in F-plasmid maintenance.
Loh S M; Cram D S; Skurray R A
Department of Microbiology, Monash University, Clayton, Victoria, Australia.
Gene (NETHERLANDS) Jun 30 **1988**, 66 (2) p259-68, ISSN 0378-1119 Journal Code: 7706761
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The leading region of the conjugative F plasmid encodes for a function, Flm, capable of extending the maintenance of normally unstable plasmids. Nucleotide sequencing and functional studies of flm locus have shown that it consists of at least two genes, flmA and **flmB** , which are physically and functionally homologous to hok and sok of parB in plasmid R1. The 52-amino acid flmA-coded polypeptide is almost identical to the hok product which has been shown to be a membrane-associated lethal protein [Gerdes et al., EMBO J. 5 (1986) 2023-2029]. Gene **flmB** codes for a 100 nucleotide, non-translated, complementary RNA which overlaps the 5' leader sequence of the flmA RNA. The flmA RNA also encodes an open reading frame (ORF70) which overlaps the flmA-coding sequence and may be a third gene involved in the Flm function. S1 analysis and functional studies suggest that the **antisense flmB** RNA binds to the flmA RNA and suppresses the expression of the lethal product, presumably by blocking coupled translation of ORF70 and flmA. Secondary structure analysis predicts that the flmA RNA is extremely stable compared to the regulatory **flmB** RNA. We suggest that when these RNA species are retained by

cells which have lost the F plasmid, the more stable flmA RNA will eventually be translated thus leading to cell death. This phenomenon provides a third mechanism, additional to ParFIA and Ccd functions, to ensure maintenance of the F plasmid in a growing bacterial population.

3/3,AB/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05888527 88313386 PMID: 2457527
Expression of c-fos **antisense** RNA inhibits the differentiation of F9 cells to parietal endoderm.
Edwards S A; Rundell A Y; Adamson E D
Cancer Research Center, La Jolla Cancer Research Foundation, California 92037.
Developmental biology (UNITED STATES) Sep **1988**, 129 (1) p91-102, ISSN 0012-1606 Journal Code: 0372762
Contract/Grant No.: HD 21957; HD; NICHD; P30 CA 30199; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
To test the putative role of c-fos in F9 differentiation, we have attempted to inhibit c-fos expression in these cells using an SV40-based expression vector (pSVneo-**sof**) that programs expression of c-fos **antisense** (**sof**) sequences as a 3' extension of a neo mRNA transcript. Of six G418-resistant clones isolated in transfection experiments, five expressed neo-**sof** transcripts. Two clones synthesized polyadenylated mRNA of the expected size (3.8 kb), two were smaller than expected, and one was larger. Two clones that expressed reduced levels of c-fos protein were inhibited in the induction of laminin, type IV collagen, and proteoglycan-19 RNA transcripts measured after 4 days of differentiation induction with RA and dibutyryl cyclic AMP. Also inhibited was the induction of the differentiation markers, TROMA-1 and TROMA-3. **Antisense** -expressing cells were not inhibited in the differentiation pathway to visceral endoderm since the alpha-fetoprotein gene was activated normally. We conclude that c-fos **antisense** expression inhibits some aspects of differentiation in F9 cells.

3/3,AB/26 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11489638 BIOSIS NO.: 199800270970
Regulation of transcription of cell division genes in the Escherichia coli dcw cluster.
AUTHOR: Vicente M(a); Gomez M J; Ayala J A
AUTHOR ADDRESS: (a)Dep. Biol. Cel. del Desarrollo, Consejo Superior Investigaciones Cientificas, Velaquez, 144, E-2**Spain
JOURNAL: CMLS Cellular and Molecular Life Sciences 54 (4):p317-324 April, **1998**
ISSN: 1420-682X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The Escherichia coli dcw cluster contains cell division genes, such as the phylogenetically ubiquitous ftsZ, and genes involved in peptidoglycan synthesis. Transcription in the cluster proceeds in the same direction as the progress of the replication fork along the chromosome. Regulation is exerted at the transcriptional and post-transcriptional levels. The absence of transcriptional termination

signals may, in principle, allow extension of the transcripts initiated at the upstream promoter (mraZ1p) even to the furthest downstream gene (envA). Complementation tests suggest that they extend into ftsW in the central part of the cluster. In addition, the cluster contains other promoters individually regulated by cis- and trans-acting signals. Dissociation of the expression of the ftsZ gene, located after ftsQ and A near the 3' end of the cluster, from its natural regulatory signals leads to an alteration in the physiology of cell division. The complexities observed in the regulation of gene expression in the cluster may then have an important biological role. Among them, LexA-binding **SOS** boxes have been found at the 5' end of the cluster, preceding promoters which direct the expression of ftsI (coding for PBP3, the penicillin-binding protein involved in septum formation). A gearbox promoter, ftsQ1p, forms part of the signals regulating the transcription of ftsQ, A and Z. It is an inversely growth-dependent mechanism driven by RNA polymerase containing sigma3, the factor involved in the expression of stationary phase-specific genes. Although the dcw cluster is conserved to a different extent in a variety of bacteria, the regulation of gene expression, the presence or absence of individual genes, and even the essentiality of some of them, show variations in the phylogenetic scale which may reflect adaptation to specific life cycles.

1998

3/3,AB/27 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11265670 BIOSIS NO.: 199800047002
Evidence of participation of McrBs in McrBC restriction in Escherichia coli K-12.
AUTHOR: Beary T P; Braymer H D; Achberger E C
AUTHOR ADDRESS: Dep. Biological Sci., Nicholls State Univ., P.O. Box 2021, Thibodaux, LA 70310**USA
JOURNAL: Journal of Bacteriology 179 (24):p7768-7775 Dec., **1997**
ISSN: 0021-9193
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The McrBC restriction system has the ability to restrict DNA containing 5-hydroxymethylcytosine, N!4-methylcytosine, and 5-methylcytosine at specific sequences. The mcrB gene produces two gene products. The complete mcrB open reading frame produces a 51-kDa protein (McrB|L) and a 33-kDa protein (McrB|S). The smaller McrB polypeptide is produced from an in-frame, internal translational start site in the mcrB gene. The McrBs sequence is identical to that of McrB|L except that it lacks 161 amino acids present at the N-terminal end of the latter protein. It has been suggested that McrB|L is the DNA binding restriction subunit. The function of McrBs is unknown, although there has been speculation that it plays some role in the modulation of McrBC restriction. Studies of the function of McrB, have been challenging since it is produced in frame with McrB|L. In this study, we tested the effects of underproduction (via **antisense** RNA) and overproduction (via gene dosage) of mcrBC gene products on restriction levels of the mcrBC!+ strain JM107. Among the parameters monitored was the induction of **SOS** responses, which indicate of DNA damage. Evidence from this study suggests that McrB|S is necessary for stabilization of the McrBC restriction complex in vivo.

1997

3/3,AB/28 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11100544 BIOSIS NO.: 199799721689
Expression of a hammerhead **ribozyme** targeted against lexA in Escherichia coli results in derepression of recA transcription.
AUTHOR: Yadava Ramesh S; Rajkumar Vincent D; Yadava Pramod K
AUTHOR ADDRESS: Applied Mol. Biol. Lab., Sch. Life Sci., Jawaharlal Nehru Univ., New Delhi**India
JOURNAL: FASEB Journal 11 (9):pA1407 **1997**
CONFERENCE/MEETING: 17th International Congress of Biochemistry and Molecular Biology in conjunction with the Annual Meeting of the American Society for Biochemistry and Molecular Biology San Francisco, California, USA August 24-29, 1997
ISSN: 0892-6638
RECORD TYPE: Citation
LANGUAGE: English
1997

3/3,AB/29 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10383327 BIOSIS NO.: 199699004472
Dependence of Mg-2+ ions of the activities of dimeric hammerhead minizymes.
AUTHOR: Amontov Sergei; Nishikawa Satoshi; Taira Kazunari(a)
AUTHOR ADDRESS: (a)National Inst. Bioscience Human Technol., AIST, MITI, 1-1 Higashi, Tsukuba Science City 305**Japan
JOURNAL: FEBS Letters 386 (2-3):p99-102 **1996**
ISSN: 0014-5793
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A minizyme is a hammerhead **ribozyme** with short oligonucleotide linkers instead of stem/loop II. In a previous study we demonstrated that a minizyme with high-level activity forms a dimeric structure with a common stem II (Amontov and Taira (1996) J. Am. Chem. **Soc**. 118, 1624-1628). We now demonstrate that the stability of the dimeric structure is influenced by Mg-2+ ions. We found that the dependence on Mg-2+ ions of the activity of homodimeric minizyme (a dimer with two identical binding sites) has composite biphasic characteristics. When the concentration of Mg-2+ ions reached a specific critical level, the dependence on the concentration of Mg-2+ ions lost its tendency to reach a plateau. In the case of the heterodimeric minizyme (a dimer with two different binding sites), we investigated the kinetic behavior of two different forms of the dimer, namely, free dimer and the complex of the dimer with an uncleavable substrate. The kinetic behavior of the free heterodimer was very similar to that of the homodimeric minizyme. In contrast, in the presence of the uncleavable substrate at a concentration as high as that of the minizyme, the curve for the dependence on Mg-2+ ions showed normal saturation kinetics. While, at low concentrations of Mg-2+ ions, the activity of the heterodimers was much higher when the dimeric structure was stabilized by the presence of the uncleavable substrate, at high concentrations of Mg-2+ ions, this difference in activity became less and less significant. Thus, high concentrations of Mg-2+ ions were able to stabilize the dimeric minizymes in the absence of the uncleavable substrate.

1996

full-length pnd mRNA was found to be translationally inactive whereas a 3'-end truncated version of it was found to be active. We have therefore suggested previously, that the 3'-end of the full-length pnd mRNA encodes a fold-back inhibitory sequence (fbi), which prevents its translation. Here we present an analysis of the metabolism of the pnd mRNAs. A mutational analysis shows that single point mutations in the fbi motif results in more rapid truncation. The fbi mutations could not be complemented by second-site mutations in either of the pndA or pndC Shine-Dalgarno (SD) elements. Surprisingly, mutations in the pndC SD element also lead to a more rapid truncation. The effect of these latter mutations was, however, complemented by mutations in a proposed anti-SD element upstream of the pndC SD. Mutations in the anti-SD element were lethal. These results show, that the pnd mRNA contains two negative control elements, one located in its very 3'-end (fbi), and one located just upstream of the pndC SD region (the anti-SD element). These observations add to the complexity of the induction scheme previously proposed to explain activation of pndA expression in plasmid-free cells: In addition to its negative effect of translation, the fbi structure also maintains a reduced processing rate in the 3'-end of the mRNA. This permits the accumulation of a reservoir of pnd mRNA, which can be activated by 3'-end processing in plasmid-free cells. The anti-SD may prevent translation of the pnd mRNA during transcription, thus preventing detrimental synthesis of toxin.

3/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08207682 94343563 PMID: 7914937
Report of a meeting: molecular basis of cancer therapy.
Gottesman M M
Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
Journal of the National Cancer Institute (UNITED STATES) Sep 7 **1994**, 86 (17) p1277-85, ISSN 0027-8874 Journal Code: 7503089
Document type: Congresses
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
There has been an explosive increase in information relevant to the pathways that determine growth signal transduction, regulation of the cell cycle, mechanism of action of oncogenes and tumor suppressors, and mechanisms of programmed cell death (apoptosis). Additional information is needed to determine the targets for anticancer therapy that are most likely to lead to cancer cell death and/or growth cessation. Current experimental clinical approaches are directed toward killing cells with unique cancer-related phenotypes, such as cell surface antigens or growth factor receptors, or altering the host immune system to attack cancer cells. The following major therapeutic targets were identified during the course of this conference: 1) Reduce activity of gene products associated with stimulation of cell growth and increase activity of gene products that inhibit growth. The major principle here is that genes known to be sufficient for malignant transformation (such as Ras, Raf, and Bcr-Abl) and genes whose expression is necessary, but not sufficient, for malignant transformation (such as some cyclins) both may be important targets for anticancer drugs. The reason genes necessary but not sufficient for cell growth are targets is that progression through the cell cycle is based on a series of "on-off" switches whose activation depends on critical levels of specific kinases and phosphatases. Subtle differences in concentration or activity of these regulators, as may be found in cancer cells, could profoundly influence the position of the switch. There are many ways to affect activity of gene products, including use of anti-sense or **ribozyme** targeting of mRNAs; manipulation of regulatory controls (i.e., state of phosphorylation of Raf and p53; effect of **SOS** and GAP

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        (c) 2003 BIOSIS
*File   5: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.

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          174281  TOXIN
          214049  TOXIC
        21931987  PY<1999
         1239258  BACTERIA
          270842  PATHOGEN
            6386  RIBOZYM?
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                  AND RIBOZYM?
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 1/3,AB/1     (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
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08592843   BIOSIS NO.: 199345010918
Regulation of tumor necrosis factor-alpha gene expression following
  ribozyme transfection: A genetically engineered model for the
  treatment of toxic shock.
AUTHOR: Kisich K O(a); Malone R W; Feldstein P A; Sipes D(a); Erickson K L
  (a); Powell J S
AUTHOR ADDRESS: (a)Dep. Cell Biol. Human Anatomy, Univ. California, Davis,
  CA 95616**USA
JOURNAL: Clinical Research  41 (2):p137A 1993
CONFERENCE/MEETING: Joint Meeting of the Association of American
Physicians, the American Society for Clinical Investigation, and the
American Federation for Clinical Research  Washington, DC, USA  April
30-May 3, 1993
ISSN: 0009-9279
RECORD TYPE: Citation
LANGUAGE: English
1993
? s (toxin or toxic) and py<1999 and ribozym?
          174281  TOXIN
          214049  TOXIC
        21931987  PY<1999
            6386  RIBOZYM?
      S2       30  (TOXIN OR TOXIC) AND PY<1999 AND RIBOZYM?
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...completed examining records
      S3       23  RD (unique items)
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 3/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10145362   99120284   PMID: 9923409
  Increased  gene  transfer into human CD34+ progenitor cells using
retroviral vectors produced by a canine packaging cell line.
  Bauer G; Sauter S; Ibanez C; Rice C R; Valdez P; Jolly D; Kohn D B
  Division  of  Research  Immunology/Bone Marrow Transplantation, Childrens
Hospital Los Angeles, CA 90027, USA.
  Biology  of  blood  and  marrow transplantation : journal of the American
Society for Blood and Marrow Transplantation (UNITED STATES)   1998,
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4 (3) p119-27, ISSN 1083-8791 Journal Code: 9600628
Contract/Grant No.: A138592; PHS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Using retroviral supernatants derived from the amphotropic murine packaging cell line PA317 and the amphotropic canine packaging cell line (DA), cord blood and mobilized peripheral blood CD34+ cells were transduced with the vector LN (neomycin resistance) and the vector L-TR/TAT neo (neomycin resistance in conjunction with a double-hammerhead **ribozyme** conferring anti-HIV activity). Different multiplicities of infection (MOI) were applied in the setup according to vector titrations on NIH-3T3 cells. PA317-based supernatants were tested at MOI of 10 and 30. Purified concentrated DA-derived vector preparations were tested at MOI of 10, 30, 100, and 300. Immediately after transduction, CD34+ cells were plated into colony assays in the presence and absence of G418 to evaluate the amount of gene transfer and potential **toxic** effects of the vectors on colony growth. The remaining cells were subjected to G418 selection in liquid culture for 12 days and subsequently challenged with HIV-1JR-FL to test for efficacy of the anti-HIV gene in macrophages derived from transduced CD34+ cells. Transduction by the PA317-packaged vectors was maximal at the lowest MOI used and did not increase with increasing MOI. In contrast, transduction by the DA-packaged vectors could be progressively increased using increased MOI. The net transduction efficiency per unit of reverse transcriptase activity in the DA vector preparations was 8.7-fold higher than in the PA317 vector supernatants. HIV-1 challenge of the cells transduced by the **ribozyme** vector derived from the PA317 packaging cells resulted in a 1.5 log inhibition of p24 output compared with the control cells containing neomycin resistance only. A 2.5 log inhibition of p24 output could be observed in the cell population transduced with DA-packaged vector supernatants. Compared with retroviral supernatants from PA317 packaging cell lines, DA packaging line-derived vector preparations demonstrated higher transduction efficiency into CD34+ cells, particularly at higher MOI, and increased efficacy of the transferred anti-HIV gene when challenged with HIV-1JR-FL. The increase in transduction efficiency may be due to a higher ratio of intact vs. defective vector particles in the DA-derived vector preparations.

3/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09945059 98374681 PMID: 9667918
The hairpin **ribozyme**: structure, assembly and catalysis.
Walter N G; Burke J M
Department of Microbiology and Molecular Genetics, Markey Center for Molecular Genetics, 306 Stafford Hall, University of Vermont, Burlington, VT 05405, USA.
Current opinion in chemical biology (ENGLAND) Feb **1998**, 2 (1) p24-30, ISSN 1367-5931 Journal Code: 9811312
Erratum in Curr Opin Chem Biol 1998 Apr;2(2) 303
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Recent studies of the hairpin **ribozyme** have revealed a distinct catalytic mechanism for this small RNA motif. Inner-sphere coordinated metal ions are not required, as the inert metal ion complex cobalt hexammine promotes catalysis. Detailed kinetic analyses have defined rates of individual steps in the catalytic cycle. Functional group modification, NMR studies of subdomains and cross-linking experiments, in combination with computer modeling, have led to a proposal for domain interactions in

the substrate-**ribozyme** complex.

3/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09889246 98316152 PMID: 9651480
Delivery of an anti-HIV-1 **ribozyme** into HIV-infected cells via cationic liposomes.
Konopka K; Rossi J J; Swiderski P; Slepushkin V A; Duzgunes N
Department of Microbiology, School of Dentistry, University of the Pacific, San Francisco, CA 94115, USA.
Biochimica et biophysica acta (NETHERLANDS) Jun 24 **1998**, 1372 (1) p55-68, ISSN 0006-3002 Journal Code: 0217513
Contract/Grant No.: AI-32399; AI; NIAID; AI-35231; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Cationic liposome-mediated intracellular delivery of a fluorescein-labeled chimeric DNA-RNA **ribozyme** targeted to the HIV-1 5' LTR was investigated, using THP-1, THP-1/HIV-1IIIB or HeLa/LAV cells. Different fluorescence patterns were observed when the cells were exposed to Lipofectamine, Lipofectin or DMRIE:DOPE (1:1) complexed to the **ribozyme**. With Lipofectamine intense cell-associated fluorescence was found. Incubation with Lipofectin resulted in less intense diffuse fluorescence, while with DMRIE an intense but sporadic fluorescence was observed. Differentiated THP-1/HIV-1IIIB cells were more susceptible to killing by liposome-**ribozyme** complexes than THP-1 cells. Under non-cytotoxic conditions (a 4-h treatment) complexes of 5, 10 or 15 microM Lipofectin or DOTAP:DOPE (1:1) and **ribozyme**, at lipid:**ribozyme** ratios of 8:1 or 4:1, did not affect p24 production in THP-1/HIV-1IIIB cells in spite of the intracellular accumulation of the **ribozyme**. A 24-h exposure of THP-1/HIV-1IIIB cells to 5 microM Lipofectin or DOTAP:DOPE (1:1) complexed with either the functional or a modified control **ribozyme** reduced virus production by approximately 30%. Thus, the antiviral effect of the liposome-complexed **ribozyme** was not sequence-specific. In contrast, the free **ribozyme** at a relatively high concentration inhibited virus production by 30%, while the control **ribozyme** was ineffective, indicating a sequence-specific effect. Both Lipofectin and DOTAP complexed with **ribozyme** were **toxic** at 10 and 15 microM after a 24-h treatment. A 4-h treatment of HeLa/LAV cells with Lipofectin at 5, 10 or 15 microM was not **toxic** to the cells, but also did not inhibit p24 production. In contrast, treatment of HeLa CD4+ cells immediately after infection with HIV-1IIIB at the same lipid concentrations and lipid:**ribozyme** ratios was cytotoxic. Our results indicate that the delivery of functional **ribozyme** into cells by cationic liposomes is an inefficient process and needs extensive improvement before it can be used in ex vivo and in vivo applications.

3/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09514282 97409672 PMID: 9264018
Ribozyme mediated targeting of cucumber mosaic virus RNA 1 and 2 in transgenic tobacco plants.
Kwon C S; Chung W I; Paek K H
Graduate School of Biotechnology, Korea University, Seoul, Korea.
Molecules and cells (KOREA) Jun 30 **1997**, 7 (3) p326-34, ISSN 1016-8478 Journal Code: 9610936

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Hammerhead **ribozymes** have been extensively used to inhibit the expression of cellular genes or viral genes mainly in the animal study. In this study, we designed a **ribozyme** targeting the conserved leader sequences of cucumber mosaic virus (CMV) RNA 1 and 2. The **ribozyme**, with asymmetric lengths of flanking complementary regions, cleaved a model substrate RNA efficiently at 26 degrees C as well as at 37 degrees C or 50 degrees C in vitro. And the **ribozyme** encoding sequence was introduced into tobacco plants and expressed with the CaMV 35S promoter and 3' NOS terminator in a monomeric type (pBIR1), tandemly repeated type (pBIR3), and cotranscriptionally combined type (pRokR) with 2.2 copies of I17N satellite RNA. Virus challenging experiments in F1 plants of respective transformants with CMV-Y showed specific reductions of viral RNA 1 and 2 in comparison with RNA 3 or 4. Although young plants of a three-leaf-stage showed rather similar mild symptom attenuations in all constructions compared to CMV-Y inoculated wild type, fully grown plants showed a differential degree of resistance upon systemic infections of CMV-Y in pRokR, pBIR3 and pBIR1 transformed plants in a decreasing order.

3/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09457779 97348446 PMID: 9204456
Expressing **ribozymes** in plants.
de Feyter R; Gaudron J
CSIRO Division of Plant Industry, Canberra, Australia.
Methods in molecular biology (Clifton, N.J.) (UNITED STATES) **1997**, 74 p403-15, ISSN 1064-3745 Journal Code: 9214969
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

3/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09322963 97214616 PMID: 9060996
Reduction of histone cytotoxicity by the Alzheimer beta-amyloid peptide precursor.
Currie J R; Chen-Hwang M C; Denman R; Smedman M; Potempska A; Ramakrishna N; Rubenstein R; Wisniewski H M; Miller D L
Laboratory of Developmental Neuromorphology, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, USA. curriejr@interport.net
Biochimica et biophysica acta (NETHERLANDS) Mar 1 **1997**, 1355 (3) p248-58, ISSN 0006-3002 Journal Code: 0217513
Contract/Grant No.: PO1-AG 04220; AG; NIA
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

In a search for Alzheimer beta-amyloid peptide precursor ligands, Potempska et al. (Arch. Biochem. Biophys. (1993) 304, 448) found that histones bind with high affinity and specificity to the secreted precursor. Because exogenous histones can be cytotoxic, we compared the effects of histones on the viability of cells which produce little beta-amyloid peptide precursor (U-937) to those on cells that produce twenty times as

much precursor (COS-7). Addition of purified histones caused necrosis of U-937 cells (histone H4, LD50 = 1.5 microM). Extracellular A beta precursor in the submicromolar range prevented histone-induced U-937 cell necrosis. Cell-surface precursor also reduced histone toxicity: COS-7 cells were less sensitive to the **toxic** effects of histone H4 (LD50 = 5.4 microM). COS-7 cells in which the expression of an APP mRNA-directed **ribozyme** reduced the synthesis of the protein by up to 80% were more sensitive to histone H4 (LD50 = 3.2 microM) than cells that expressed the vector alone. Histone H4 binds to cell-associated A beta precursor. Cells expressing the A beta precursor-directed **ribozyme** bound less 125I-labeled histone H4 than those expressing the vector alone. In the limited extracellular space of tissues in vivo, both secreted and cell-surface A beta precursor protein may play significant roles in trapping chromatin or histones and removing them from the extracellular milieu.

3/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09230862 97141440 PMID: 8986637
Toxicity of cationic lipid-**ribozyme** complexes in human prostate tumor cells can mimic **ribozyme** activity.
Freedland S J; Malone R W; Borchers H M; Zadourian Z; Malone J G; Bennett M J; Nantz M H; Li J H; Gumerlock P H; Erickson K L
Department of Medical Pathology, University of California, Davis, California, 95616, USA.
Biochemical and molecular medicine (UNITED STATES) Dec **1996**, 59 (2) p144-53, ISSN 1077-3150 Journal Code: 9508702
Contract/Grant No.: CA 47050; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Prostate tumor cell lines have been shown to both produce interleukin-6 (IL-6) and express the IL-6 receptor, suggesting a potential autocrine growth regulatory role for IL-6. We explored the role of IL-6 in the proliferation of the human prostatic carcinoma cell line, DU145, using **ribozymes** to inhibit IL-6 expression. Hammerhead-type **ribozymes** targeted against IL-6 mRNA sequences were prepared, and in vitro analyses were used to demonstrate that these molecules catalyzed the cleavage of IL-6 mRNA poly- nucleotide fragments. To test in situ activity, these **ribozymes** were transfected into DU145 cells using cationic transfection lipids, cytofectins. Treatment of cultured cells with **ribozyme** /cationic lipid complexes resulted in a reduction of IL-6 protein levels in the supernatant and reduced numbers of DU145 cells 48 h after treatment. However, similar results were also seen following treatment with control RNA/lipid complexes. This reduction in IL-6 levels and cell numbers was a function of the RNA/lipid complexes and was not seen with either lipid or RNA alone. Therefore, the reductions in IL-6 levels and cell numbers observed were not due to **ribozyme**-mediated cleavage of IL-6 mRNA, but rather reflected a dose-dependent, nonspecific **toxic** effect of the treatment with **ribozyme** /cytofectin complexes. This effect can resemble functional **ribozyme** activity, complicating analysis of the activity of synthetic **ribozymes** after transfection into cultured cells.

3/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08959925 96313822 PMID: 8703987
Human immunodeficiency virus type-1 (HIV-1) infection increases the

sensitivity of macrophages and THP-1 cells to cytotoxicity by cationic liposomes.

Konopka K; Pretzer E; Felgner P L; Duzgunes N

Department of Microbiology, University of the Pacific School of Dentistry, San Francisco, CA 94115, USA.

Biochimica et biophysica acta (NETHERLANDS) Jul 24 **1996**, 1312 (3) p186-96, ISSN 0006-3002 Journal Code: 0217513

Contract/Grant No.: RO1 AI-32399; AI; NIAID; UO1 AI-35231; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Cationic liposomes may be valuable for the delivery of anti-sense oligonucleotides, **ribozymes** , and therapeutic genes into human immunodeficiency virus type 1 (HIV-1)-infected and uninfected cells. We evaluated the toxicity of three cationic liposomal preparations, Lipofectamine, Lipofectin, and 1, 2-dimyristyloxypropyl-3-dimethyl-hydroxye thyl ammonium bromide (DMRIE) reagent, to HIV-infected and uninfected cells. Monocyte/macrophages were infected with HIV-1BaL and treated with liposomes in medium containing 20% fetal bovine serum (FBS) for 4 h or 24 h at 37 degree C. Uninfected monocytic THP-1 cells and chronically infected THP-1/HIV-1IIIB cells were treated with phorbol 12-myristate 13-acetate (PMA) and exposed to liposomes in the presence of 10% FBS. Toxicity was evaluated by the Alamar Blue assay and viral p24 production. The **toxic** effect of cationic liposomes was very limited with uninfected cells, although concentrations of liposomes that were not **toxic** within a few days of treatment could cause toxicity at later times. In HIV-1BaL-infected macrophages, Lipofectamine (up to 8 microM) and Lipofectin (up to 40 microM) were not **toxic** after a 4-h treatment, while DMRIE reagent at 40 microM was **toxic**. While a 4-h treatment of THP-1/HIV-1IIIB cells with the cationic liposomes was not **toxic**, even up to 14 days post-treatment, all three cationic liposomes were **toxic** to cells at the highest concentration tested after a 24-h treatment. Similar results were obtained with the Alamar Blue assay, Trypan Blue exclusion and a method that enumerates nuclei. Infected cells with relatively high overall viability could be impaired in their ability to produce virions, indicating that virus production appears to be more sensitive to treatment with the cationic liposomes than cell viability. Our results indicate that HIV-infected cells are more susceptible than uninfected cells to killing by cationic liposomes. The molecular basis of this differential effect is unknown; it is proposed that alterations in cellular membranes during virus budding cause enhanced interactions between cationic liposomes and cellular membranes.

3/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08944903 96298796 PMID: 8755166

Strategies for the suppression of peroxidase gene expression in tobacco. II. In vivo suppression of peroxidase activity in transgenic tobacco using **ribozyme** and antisense constructs.

McIntyre C L; Bettenay H M; Manners J M

CSIRO Division of Tropical Crops and Pastures, St. Lucia, Australia.

Transgenic research (ENGLAND) Jul **1996**, 5 (4) p263-70, ISSN 0962-8819 Journal Code: 9209120

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Several strategies involving the use of antisense and **ribozyme** constructs in different expression vectors were investigated as methods of suppressing gene expression in planta. We had previously identified an

efficiently cleaving **ribozyme** (Rz), with two catalytic units and 60 nucleotide (nt) of complementary sequence, to the lignin-forming peroxidase of tobacco (TPX). This Rz was cloned behind the 35S CaMV (35S) and nopaline synthase (NOS) promoters, and into a vector utilising the tobacco tyrosine tRNA for expression. For comparison with more traditional antisense strategies, full-length TPX antisense (AS) constructs were also constructed behind the NOS and 35S promoters. Populations of transgenic tobacco containing these constructs were produced and compared to control plants transformed with the vector only. Significant suppression of peroxidase expression in the range of 40-80% was seen in the T0 and T1 populations carrying 35S-AS, 35S-Rz and tRNA-Rz constructs. Co-segregation of the suppressed peroxidase phenotype and the tRNA-Rz transgenes was demonstrated. Northern blot analysis indicated that levels of TPX mRNA were lower in the Rz plants. No evidence of mRNA cleavage was observed and thus it was unclear if the Rz constructs were acting as Rzs in vivo. Transgenic plants containing the tRNA-Rz construct had significantly lower levels of peroxidase than the other transgenic plants. There was no significant difference in levels of suppression of TPX between the short Rz in the 35S vector and the full-length AS constructs. Although peroxidase levels were significantly reduced in transgenic plants carrying 35S-AS, 35S-Rz and tRNA-Rz constructs, no significant difference in lignin levels was observed.

3/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08944902 96298795 PMID: 8755165
Strategies for the suppression of peroxidase gene expression in tobacco. I. Designing efficient **ribozymes**.
McIntyre C L; Manners J M
CSIRO Division of Tropical Crops and Pastures, St. Lucia, Australia.
Transgenic research (ENGLAND) Jul **1996**, 5 (4) p257-62, ISSN 0962-8819 Journal Code: 9209120
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Five short hammerhead **ribozymes** (Rzs) were constructed and tested, using a range of in vitro reaction conditions, for catalytic activity against the mRNA encoding the lignin-forming peroxidase (TPX) of tobacco. Although all 5 Rzs were shown to be able to cleave the RNA substrate, percentage cleavage varied with pre-denaturation of Rz and substrate, incubation temperature, length of incubation and **ribozyme** (Rz)-to-substrate ratio. One Rz with two catalytic units and 60 nucleotides of complementary sequence in 3 regions was shown to most efficiently cleave the substrate under all in vitro conditions tested. This **ribozyme** cleaved better than the two single **ribozymes** from which it was made. The superior cleaving ability of this Rz was shown to be due to the accessibility of the chosen target site and to the increased length of the hybridizing arms spanning this accessible region of the RNA.

3/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08823935 96180650 PMID: 8602148
A **ribozyme** gene and an antisense gene are equally effective in conferring resistance to tobacco mosaic virus on transgenic tobacco.
de Feyter R; Young M; Schroeder K; Dennis E S; Gerlach W
CSIRO Division of Plant Industry, Canberra, Australia.
Molecular & general genetics : MGG (GERMANY) Feb 25 **1996**, 250

A chimeric gene encoding a **ribozyme** under the control of the cauliflower mosaic virus (CaMV) 35S promoter was introduced into transgenic tobacco plants. In vivo activity of this **ribozyme**, which was designed to cleave npt mRNA, was previously demonstrated by transient expression assays in plant protoplasts. The **ribozyme** gene was transferred into transgenic tobacco plants expressing an rbcS-npt chimeric gene as an indicator. Five double transformants out of sixteen exhibited a reduction in the amount of active NPT enzyme. To measure the amount of **ribozyme** produced, in the absence of its target, the **ribozyme** and target genes were separated by genetic segregation. The steady-state concentrations of **ribozyme** and target RNA were shown to be similar in the resulting single transformants. Direct evidence for a correlation between reduced npt gene expression and **ribozyme** expression was provided by crossing a plant containing only the **ribozyme** gene with a transgenic plant expressing the npt gene under control of the 35S promoter, i.e. the same promoter used to direct **ribozyme** expression. The expression of npt was reduced in all progeny containing both transgenes. Both steady-state levels of npt mRNA and amounts of active NPT enzyme are decreased. In addition, our data indicate that, at least in stable transformants, a large excess of **ribozyme** over target is not a prerequisite for achieving a significant reduction in target gene expression.

3/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08289688 95047470 PMID: 7958987

A stable hammerhead structure is not required for endonucleolytic activity of a **ribozyme** in vivo.

Steinecke P; Steger G; Schreier P H

Max-Planck-Institut fur Zuchtungsforschung, Abteilung Genetische Grundlagen der Pflanzenzuchtung, Koln, Germany.

Gene (NETHERLANDS) Nov 4 **1994**, 149 (1) p47-54, ISSN 0378-1119 Journal Code: 7706761

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Cleavage of a specific target, the mRNA encoding the bacterial neomycin phosphotransferase, by mutant satellite RNA of subterranean clover mottle virus (sSCMoV) **ribozymes** (Rz) was used to study the role of the hammerhead (Hh) structure in Rz activity in cis and in trans. The bimolecular Rz-target RNA interaction was predicted by computer secondary structure analysis. In vivo, endonucleolytic cleavage was determined in plant protoplasts and compared with in vitro results. Two point mutations within the Hh were studied in detail. A Rz mutant with a point mutation in the most distal nucleotide of the catalytic domain (A14G) showed no endonucleolytic activity in vivo. A second point mutation inside helix II (G11.3C) which destabilizes the helix and, according to thermodynamic calculations, should disrupt the conserved Hh structure, unexpectedly displayed Rz activity in trans in vivo. In vitro, this mutant exhibited an activity similar to the wild-type Rz in cis, but no significant activity in trans. It therefore appears that helix II within the Rz Hh structure is not required in vivo for endonucleolytic activity, nor for stability of the Rz transcript, and that in vitro results are inadequate to predict Rz activity in living cells.

3/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07973788 94115156 PMID: 8286926
A **ribozyme** that enhances gene suppression in tobacco protoplasts.
Perriman R; Graf L; Gerlach W L
CSIRO Division of Plant Industry, Canberra, Australia.
Antisense research and development (UNITED STATES) Fall **1993**, 3 (3) p253-63, ISSN 1050-5261 Journal Code: 9110698
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Site-directed mutagenesis has been used to produce two hammerhead **ribozyme** molecules targeting the chloramphenicol acetyltransferase gene (CAT). One **ribozyme** has a single catalytic domain between two 12-nucleotide arms that can hybridize 5' and 3' of the GUC target site of the CAT RNA transcript. The second **ribozyme** is a full-length antisense RNA with four catalytic domains inserted along the length, each targeting a specific GUC site within the CAT mRNA. Our results show that both **ribozymes** can produce almost equivalent rates of cleavage of the CAT mRNA in vitro (T1/2 of 18 or 15 min, respectively). In tobacco protoplasts we show consistently greater gene suppression in the presence of the long **ribozyme** molecule, compared with the equivalent antisense (22% gene reduction for antisense compared with 44% with the long **ribozyme**). These results suggest that hammerhead **ribozymes** may be developed for the inactivation of gene activity in plant cells.

3/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07766449 93293951 PMID: 8514842
Transfection of whole plants from wounds inoculated with Agrobacterium tumefaciens containing cDNA of tobacco mosaic virus.
Turpen T H; Turpen A M; Weinzettl N; Kumagai M H; Dawson W O
Biosource Genetics Corporation, Vacaville, CA 95688.
Journal of virological methods (NETHERLANDS) May **1993**, 42 (2-3) p227-39, ISSN 0166-0934 Journal Code: 8005839
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We engineered cDNA of tobacco mosaic tobamovirus (TMV) into Agrobacterium tumefaciens for inoculation of plant cells. The resulting bacterial strains were used to transfect tobacco (Nicotiana tabacum cv. Xanthi and Xanthi/nc) with wild type and a defective virus. Lesion formation on Xanthi/nc tobacco was used to measure the timing and efficiency of transfection. Infections mediated by Agrobacterium produced lesions an average of two days later than infections produced by inoculation with virions. The addition of approximately 80 bp of non-viral sequences to the 5'-end of TMV transcripts abolished transfection. Transcripts with non-viral sequences at the 3'-end initiated infections, while precise transcript termination with a synthetic **ribozyme** sequence increased transfection frequencies two-fold. Culture conditions reported to induce genes of the vir region of the Agrobacterium Ti plasmid also increased the transfection frequency approximately two-fold. Therefore, in addition to the pararetroviruses and geminiviruses previously described, 'agroinoculation' may be used to infect plants with plus-sense RNA viruses.

3/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07735326 93261836 PMID: 7684131

Catalytically active geometry in the reversible circularization of 'mini-monomer' RNAs derived from the complementary strand of tobacco ringspot virus satellite RNA.

Feldstein P A; Bruening G
Department of Plant Pathology, College of Agricultural and Environmental Sciences, University of California, Davis 95616.

Nucleic acids research (ENGLAND) Apr 25 **1993**, 21 (8) p1991-8, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The less abundant polarity of the satellite RNA of tobacco ringspot virus, designated sTobRV(-)RNA, contains a **ribozyme** and its substrate. We demonstrate that the **ribozyme** can catalyze the ligation of substrate cleavage products and that oligoribonucleotides, termed 'mini-monomers' and containing little more than covalently attached **ribozyme** and substrate cleavage products, circularized spontaneously, efficiently and reversibly. The kinetics of ligation and cleavage of one such mini-monomer was consistent with a simple unimolecular reaction at some temperatures. Evidence suggests that the circular ligation product includes a 5 bp stem that is connected to a 4 bp stem by a bulge loop. Reduction of the bulge loop to one nt is expected to place the 4 and 5 bp helices in a nearly coaxial, rather than an angled or parallel, orientation. Such molecules did not circularize in a unimolecular reaction but did when incubated with second, trans-acting oligoribonucleotides that had either the original or a substituted 4 bp helix. These results suggest that a bulge loop that is too small prevents formation of geometry essential for unimolecular ligation. We suggest the term 'paperclip' to represent the arrangement of RNA strands in the region of sTobRV(-)RNA that participates in the cleavage and ligation reactions.

3/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07298074 92224891 PMID: 1373377

Expression of a chimeric **ribozyme** gene results in endonucleolytic cleavage of target mRNA and a concomitant reduction of gene expression in vivo.

Steinecke P; Herget T; Schreier P H
Max-Planck-Institut fur Zuchtungsforschung, Koln, FRG.

EMBO journal (ENGLAND) Apr **1992**, 11 (4) p1525-30, ISSN 0261-4189 Journal Code: 8208664
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The subclass of catalytic RNAs termed **ribozymes** cleave specific target RNA sequences in vitro. Only circumstantial evidence supports the idea that **ribozymes** may also act in vivo. In this study, **ribozymes** with a hammerhead motif directed against a target sequence within the mRNA of the neomycin phosphotransferase gene (npt) were embedded into a functional chimeric gene. Two genes, one containing the **ribozyme** and the other producing the target, were cotransfected into plant protoplasts. Following in vivo expression, a predefined cleavage product of the target mRNA was detected by ribonuclease protection. Expression of both the **ribozyme** gene and the target gene was driven by the CaMV 35S promoter. Concomitant with the endonucleolytic cleavage of the target mRNA, a complete reduction of NPT activity was observed. An A to G substitution within the **ribozyme** domain completely inactivates **ribozyme** -mediated hydrolysis but still shows a reduction in NPT activity, albeit less pronounced. Therefore, the reduction of NPT activity

produced by the active **ribozyme** is best explained by both hydrolytic cleavage and an antisense effect. However, the mutant **ribozyme** --target complex was more stable than the wildtype **ribozyme**--target complex. This may result in an overestimation of the antisense effect contributing to the overall reduction of gene expression.

3/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07258886 92186505 PMID: 1347590
Gene therapy for cancer.
Gutierrez A A; Lemoine N R; Sikora K
Department of Clinical Oncology, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK.
Lancet (ENGLAND) Mar 21 **1992**, 339 (8795) p715-21, ISSN 0140-6736 Journal Code: 2985213R
Comment in Lancet. 1992 May 2;339(8801) 1109-10; Comment in PMID 1349118
Document type: Journal Article; Review; Review, Academic
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The molecular basis of cancer is now understood to involve activation of dominant oncogenes and inactivation of tumour suppressor genes, and these genetic events may represent novel targets for cancer therapy. This review focuses on the potential use and ethical implications of gene transfer to alter the behaviour of somatic cells in cancer patients. Antisense nucleic acids and **ribozymes** represent informational drugs that may be used to modulate the expression of selected genes and suppress malignant behaviour in cancer cells. Genetic immunomodulation by introducing genes for cytokines into cancer cells or lymphocytes can stimulate a cytotoxic immune response against the tumour. Gene transfer techniques can be applied to target prodrug activation specifically to tumour cells and also to protect normal tissues against **toxic** chemotherapy. Gene replacement therapy could even be used to restore the function of defective tumour suppressor genes.

3/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07223194 92148918 PMID: 1738195
Ribozymes which cleave arenavirus RNAs: identification of susceptible target sites and inhibition by target site secondary structure.
Xing Z; Whitton J L
Department of Neuropharmacology, Scripps Research Institute, La Jolla, California 92037.
Journal of virology (UNITED STATES) Mar **1992**, 66 (3) p1361-9, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: AG-04342; AG; NIA; AI-27028; AI; NIAID; NS-12428; NS; NINDS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The development of safe and effective antiviral agents has been a slow process, largely because of the difficulty in distinguishing between virus and host functions; materials **toxic** to the virus are frequently harmful also to the host in which the agent resides. Recently, techniques which target nucleic acid sequences as a means of reducing gene expression have emerged. This antisense armamentarium includes **ribozymes**, RNA enzymes which cleave other RNA molecules in a sequence-specific manner. We

wish to assess the ability of **ribozymes** to control animal virus infection. Reasoning that the viruses most vulnerable to **ribozyme** intervention will be those whose complete life cycle is based on RNA (with no DNA stage), we have begun to develop **ribozymes** directed toward lymphocytic choriomeningitis virus (LCMV), the prototype of the arenavirus family. Using **ribozymes** of the hammerhead variety, we have identified several sites on the LCMV genome which can be efficiently cleaved in trans. The efficiency of cleavage is site dependent, and we demonstrate that secondary structure at the target site can abolish **ribozyme** cleavage. Computer-assisted analysis indicates that much of the LCMV genome may be involved in base pairing, which may render it similarly resistant to **ribozyme** attack. The few remaining open regions of LCMV lack a GUC target site, on which most studies to date have relied. Here we show that AUC, CUC, and AUU are alternative sites which can be cleaved by trans-acting **ribozymes** . This finding is important given the aforementioned restriction of available sites, imposed by secondary structure.

3/3,AB/23 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08592843 BIOSIS NO.: 199345010918
Regulation of tumor necrosis factor-alpha gene expression following **ribozyme** transfection: A genetically engineered model for the treatment of **toxic** shock.
AUTHOR: Kisich K O(a); Malone R W; Feldstein P A; Sipes D(a); Erickson K L (a); Powell J S
AUTHOR ADDRESS: (a)Dep. Cell Biol. Human Anatomy, Univ. California, Davis, CA 95616**USA
JOURNAL: Clinical Research 41 (2):p137A **1993**
CONFERENCE/MEETING: Joint Meeting of the Association of American Physicians, the American Society for Clinical Investigation, and the American Federation for Clinical Research Washington, DC, USA April 30-May 3, 1993
ISSN: 0009-9279
RECORD TYPE: Citation
LANGUAGE: English
1993

1993
? s pathogen and py<1999 and ribozym?
270842 PATHOGEN
21931987 PY<1999
6386 RIBOZYM?
S4 64 PATHOGEN AND PY<1999 AND RIBOZYM?
? rd
...examined 50 records (50)
...completed examining records
S5 58 RD (unique items)
? s s5 and promoter? and vector?
58 S5
201825 PROMOTER?
213967 VECTOR?
S6 1 S5 AND PROMOTER? AND VECTOR?
? t s6/3,ab/all

6/3,AB/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10989224 BIOSIS NO.: 199799610369
Intracellular inhibition of HIV-1 replication using a dual protein- and RNA-based strategy.
AUTHOR: Duan L(a); Zhu M; Ozaki I; Zhang H; Wei D L; Pomerantz R J
AUTHOR ADDRESS: (a)Dorrance H. Hamilton Lab., Cent. Human Virol., Div. Infect. Dis., Dep. Med., Jefferson Med. Coll**USA
JOURNAL: Gene Therapy 4 (6):p533-543 **1997**
ISSN: 0969-7128
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Exporting unspliced human immunodeficiency virus type 1 (HIV-1) RNA from the nucleus to the cytoplasm, through an interaction between the viral regulatory Rev protein and Rev response element (RRE) RNA, is a critical step in the HIV-1 life-cycle. Disruption of either Rev or the RRE will completely inhibit HIV-1 replication. As such, a strategy for somatic gene therapy to treat HIV-1 infection by intracellular expression of an anti-HIV-1 Rev single chain variable fragment (SFv) and a **ribozyme** which specifically targets the RRE was developed. The anti-Rev D8SFv, which specifically targets the Rev activation domain, may be a key component of combination intracellular immunization, as it has been previously shown to potently inhibit Rev function, thereby inhibiting viral replication. In the present studies, different HIV-1 RRE region-specific hammerhead **ribozymes** were constructed and their anti-HIV-1 replication effects were assayed in diverse RNA polymerase (pol) II and III **promoters** and **vector** systems in cell culture. Utilizing this combination of an SFv and a **ribozyme** as a dual strategy to block HIV-1 replication, both at the protein and RNA level, data from these studies demonstrated that potent inhibition of HIV-1 replication can be achieved via this approach. Combination gene therapies hold promise, analogous to combination chemotherapeutic regimens, for the in vivo treatment of HIV-1 infections.

1997
? ds

Set	Items	Description
S1	1	(TOXIN OR TOXIC) AND PY<1999 AND (BACTERIA OR PATHOGEN) AND RIBOZYM?
S2	30	(TOXIN OR TOXIC) AND PY<1999 AND RIBOZYM?
S3	23	RD (unique items)
S4	64	PATHOGEN AND PY<1999 AND RIBOZYM?
S5	58	RD (unique items)

S6 1 S5 AND PROMOTER? AND VECTOR?
? s s5 and cassette
58 S5
13889 CASSETTE
S7 0 S5 AND CASSETTE
? s s5 and (viroid? or phage? or bacter?)
58 S5
1994 VIROID?
73875 PHAGE?
1685651 BACTER?
S8 6 S5 AND (VIROID? OR PHAGE? OR BACTER?)
? rd
...completed examining records
S9 6 RD (unique items)
? t s9/3,ab/all

9/3,AB/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11836246 BIOSIS NO.: 199900082355
Antisense RNA and **ribozyme**-mediated resistance to plant viruses.
BOOK TITLE: Plant virus disease control
AUTHOR: Tabler M(a); Tsagris M; Hammond J
BOOK AUTHOR/EDITOR: Hadidi A; Khetarpal R K; Koganezawa H: Author
AUTHOR ADDRESS: (a)Found. Res. and Technol.-Hellas, Inst. Mol. Biol. and Biotechnol., P.O. Box 1527, GR-711 10 Hera**Greece
p79-93 **1998**
BOOK PUBLISHER: American Phytopathological Society (APS) Press, 3340 Pilot Knob Road, St. Paul, Minnesota 55121, USA
ISBN: 0-89054-191-4
DOCUMENT TYPE: Book
RECORD TYPE: Citation
LANGUAGE: English
1998

9/3,AB/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11796909 BIOSIS NO.: 199900043018
Viroids: Tkhe noncoding genomes.
AUTHOR: Flores Ricardo(a); Di Serio Francesco; Hernandez Carmen
AUTHOR ADDRESS: (a)Inst. Biol. Mol. Celular Plantas, Univ. Politecnica Valencia, Camino de Vera 14, 46022 Valencia**Spain
JOURNAL: Seminars in Virology 8 (1):p65-73 Feb., **1997**
ISSN: 1044-5773
DOCUMENT TYPE: Literature Review
RECORD TYPE: Citation
LANGUAGE: English
1997

9/3,AB/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11730409 BIOSIS NO.: 199800512140
A human liver cell line exhibits efficient translation of HCV RNAs produced by a recombinant adenovirus expressing T7 RNA polymerase.
AUTHOR: Aoki Yoichiro; Aizaki Hideki; Shimoike Takashi; Tani Hideki; Ishii Koji; Saito Izumu; Matsuura Yoshiharu; Miyamura Tatsuo(a)
AUTHOR ADDRESS: (a)Dep. Virol. II, Natl. Inst. Infectious Dis., 1-23 1

Toyama, Shinjuku-ku, TRokyo 162-8640**Japan
JOURNAL: Virology 250 (1):p140-150 Oct. 10, **1998**
ISSN: 0042-6822
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: An in vitro system that supports the efficient growth of hepatitis C virus (HCV) and reflects its complete in vitro replication cycle has not yet been established. The establishment of a minigene RNA of HCV in mammalian cells could facilitate the study of virus-cell interactions and the molecular pathogenesis of this virus. We constructed a replication-deficient recombinant adenovirus expressing **bacteriophage** T7 RNA polymerase under the control of CAG promoter (AdexCAT7). A high level of T7 RNA polymerase was detectable for at least 11 days after inoculation. Cells infected with AdexCAT7 were then transfected with plasmids carrying the authentic T7 promoter, the 5' untranslated region (UTR) of encephalomyocarditis virus, a luciferase gene, and a T7 terminator (pT7EMCVLuc) or carrying the modified T7 promoter, the 5'UTR of HCV, a luciferase gene, the coding region of C-terminal of NS58 and the 3'UTR of HCV, a **ribozyme** of hepatitis D virus and a T7 terminator (pT7HCVLuc). Most of the cell lines examined supported a higher expression of luciferase by transfection with pT7EMCVLuc than with pT7HCVLuc. However, one cell line, FLC4, derived from a human hepatocellular carcinoma, exhibited very high reporter gene expression with pT7HCVLuc. In this cell line, transfection with RNA synthesized In vitro from pT7HCVLuc induced a higher level of reporter gene expression than RNA from pT7EMCVLuc. The T7-adenovirus system for the synthesis of HCV minigenes in vivo provides useful information on the molecular mechanisms of HCV translation in human liver cells.

1998

9/3,AB/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11178499 BIOSIS NO.: 199799799644
Chrysanthemum chlorotic mottle **viroid**: Unusual structural properties of a subgroup of self-cleaving **viroids** with hammerhead **ribozymes**.
AUTHOR: Navarro Beatriz; Flores Ricardo(a)
AUTHOR ADDRESS: (a)Inst. Biol. Molecular Celular Plantas, UPV-CSIC, Univ. Politecnia Valencia, Camino Vera 14, 4602**Spain
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 94 (21):p11262-11267 **1997**
ISSN: 0027-8424
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The causal agent of chrysanthemum chlorotic mottle (CChM) disease has been identified, cloned, and sequenced. It is a **viroid** RNA (CChMVd) of 398-399 nucleotides. In vitro transcripts with the complete CChMVd sequence were infectious and induced the typical symptoms of the CChM disease. CChMVd can form hammerhead structures in both polarity strands. Plus and minus monomeric CChMVd RNAs self-cleaved during in vitro transcription and after purification as predicted by these structures, which are stable and most probably act as single hammerhead structures as in peach latent mosaic **viroid** (PLMVd), but not in avocado sunblotch **viroid** (ASBVd). Moreover, the plus CChMVd hammerhead structure also appears to be active in vivo, because the 5' terminus of the linear plus CChMVd RNA isolated from infected tissue is that predicted by the corresponding hammerhead **ribozyme**. Both

hammerhead structures of CChMVd display some peculiarities: the plus self-cleaving domain has an unpaired "A after the conserved A9 residue, and the minus one has an unusually long helix II. The most stable secondary structure predicted for CChMVd is a branched conformation that does not fulfill the rod-like or quasi-rod-like model proposed for the in vitro structure of most **viroids** with the exception of PLMVd, whose proposed secondary structure of lowest free energy also is branched. The unusual conformation of CChMVd and PLMVd is supported by their insolubility in 2 M LiCl, in contrast to ASBVd and a series of representative non-self-cleaving **viroids** that are soluble under the same high salt conditions. These results support the classification of self-cleaving **viroids** into two subgroups, one formed by ASBVd and the other one by PLMVd and CChMVd.

1997

9/3,AB/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10988998 BIOSIS NO.: 199799610143
The self-catalytic RNA motifs of the hepatitis delta virus.
AUTHOR: Mercure Stephane; Lafontaine Daniel; Roy Guylaine; Perreault Jean-Pierre(a)
AUTHOR ADDRESS: (a)Univ. Sherbrooke, Dep. Biochim., Sherbrooke, PQ J1H 5N4**Canada
JOURNAL: M-S (Medecine Sciences) 13 (5):p662-669 **1997**
ISSN: 0767-0974
DOCUMENT TYPE: Literature Review
RECORD TYPE: Abstract
LANGUAGE: French; Non-English
SUMMARY LANGUAGE: French; English

ABSTRACT: The hepatitis delta virus (HDV) is responsible for a common form of viral hepatitis. This virus is unique among human pathogens and shares several characteristics with plant **viroids**, such as their DNA-independent rolling circle replication mechanism and a highly folded small circular RNA genome. The present review will focus on the structures, the molecular mechanism and the applications of the delta **ribozyme** which is the self-catalytic motif found in HDV. This motif performs the cis cleavage of the multimeric strands in monomers during HDV replication. The delta **ribozyme** may also be modified so cleavage of a target RNA is achieved in trans. This last property might be helpful in the development of therapeutic applications aimed at inhibiting replication of viral RNA or translation of target mRNA.

1997

9/3,AB/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10950055 BIOSIS NO.: 199799571200
Ribozyme-mediated high resistance against potato spindle tuber **viroid** in transgenic potatoes.
AUTHOR: Yang Xicai; Yie Yin; Zhu Feng; Liu Yule; Kang Liangyi; Wang Xiaofeng; Tien Po(a)
AUTHOR ADDRESS: (a)Inst. Microbiol., Chinese Academy Sci., Beijing 100080**China
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 94 (10):p4861-4865 **1997**
ISSN: 0027-8424

RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A hammerhead **ribozyme** (R(-)) targeting the minus strand RNA of potato spindle tuber **viroid** (PSTVd) and a mutated nonfunctional **ribozyme** (mR(-)) were designed, cloned, and transcribed. As predicted, both monomer and dimer transcripts of the active R(-) **ribozyme** gene could cleave the PSTVd minus strand dimer RNA into three fragments of 77, 338, and 359 bases in vitro at 25 and 50 degree C. The tandem dimer genes of R(-) and mR(-) were subcloned separately into the plant expression vector pROK2. Transgenic potato plants (cultivar Desiree) were generated by Agrobacterium tumefaciens-mediated transformation. Twenty-three of 34 independent transgenic plant lines expressing the active **ribozyme** R(-) resulted in having high levels of resistance to PSTVd, being free of PSTVd accumulation after challenge inoculation with PSTVd, but the remaining lines showed weaker levels of resistance to PSTVd with low levels of PSTVd accumulation. In contrast, 59 of 60 independent transgenic lines expressing the mutated **ribozyme** mR(-) were susceptible to PSTVd inoculation and had levels of PSTVd accumulation similar to that of the control plants transformed with the empty vector. The resistance against PSTVd replication was stably inherited to the vegetative progenies.

1997

(3) p329-38, ISSN 0026-8925 Journal Code: 0125036
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Ribozymes of the hammerhead class can be designed to cleave a target RNA in a sequence-specific manner and can potentially be used to specifically modulate gene activity. We have targeted the tobacco mosaic virus (TMV) genome with a **ribozyme** containing three catalytic hammerhead domains embedded within a 1 kb antisense RNA. The **ribozyme** was able to cleave TMV RNA at all three target sites in vitro at 25 degrees C. Transgenic tobacco plants were generated which expressed the **ribozyme** or the corresponding antisense constructs directed at the TMV genome. Six of 38 independent transgenic plant lines expressing the **ribozyme** and 6 of 39 plant lines expressing the antisense gene showed some level of protection against TMV infection. Homozygous progeny of some lines were highly resistant to TMV; at least 50% of the plants remained asymptomatic even when challenged with high levels of TMV. These plants also displayed resistance to infection with TMV RNA or the related tomato mosaic virus (ToMV). In contrast, hemizygous plants of the same lines displayed only very weak resistance when inoculated with low amounts of TMV and no resistance against high inoculation levels. Resistance in homozygous plants was not overcome by a TMV strain which was altered at the three target sites to abolish **ribozyme**-mediated cleavage, suggesting that the **ribozyme** conferred resistance primarily by an antisense mechanism.

3/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08735297 96090688 PMID: 8531816
Ribozymes.
Steinecke P; Schreier P H
Department of Genetic Principles of Plant Breeding Max-Planck-Institut fur Zuchtungsforschung, Koln, Germany.
Methods in cell biology (UNITED STATES) **1995**, 50 p449-60, ISSN 0091-679X Journal Code: 0373334
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

3/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08581489 95346701 PMID: 7621251
Papillomaviruses as targets for cancer gene therapy.
Shillitoe E J; Kamath P; Chen Z
Department of Microbiology and Immunology, SUNY College of Medicine, Syracuse 13210, USA.
Cancer gene therapy (UNITED STATES) Sep **1994**, 1 (3) p193-204, ISSN 0929-1903 Journal Code: 9432230
Contract/Grant No.: DE 10842; DE; NIDCR
Document type: Journal Article; Review; Review, Academic
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Gene therapy of human cancer is likely to be most effective when it is directed at targets that are expressed in cancer cells but are lacking from other cells. Human papillomaviruses can provide such targets, since these

viruses are present in many cervical and oral cancers, and are likely to be etiological agents of the tumor. Continued expression of human papillomavirus genes is probably necessary for the growth of these cancers, and effective gene therapy could consist of antisense or **ribozyme** molecules directed against these genes. Some human papillomavirus gene products are antigenic, and immunotherapy based upon these antigens might prove clinically beneficial. Human papillomaviruses have specific promoters, are linked to **toxin** genes, the **toxin** may be selectively expressed by tumor cells where the virus genes are active. Thus, there are several approaches for the development of specific gene therapy for human cancers that contain human papillomaviruses.

3/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08562800 95320234 PMID: 7597097
Effective **ribozyme** delivery in plant cells.
Perriman R; Bruening G; Dennis E S; Peacock W J
Center for Engineering Plants Resistant Against Pathogens (CEPRAP), University of California, Davis 95616, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Jun 20 **1995**, 92 (13) p6175-9, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Hammerhead **ribozyme** sequences were incorporated into a tyrosine tRNA (tRNA(Tyr)) and compared with nonembedded molecules. To increase the levels of **ribozyme** and control antisense in vivo, sequences were expressed from an autonomously replicating vector derived from African cassava mosaic geminivirus. In vitro, the nonembedded **ribozyme** cleaved more target RNA, encoding chloramphenicol acetyltransferase (CAT), than the tRNA(Tyr) **ribozyme**. In contrast, the tRNA(Tyr) **ribozyme** was considerably more effective in vivo than either the nonembedded **ribozyme** or antisense sequences, reducing CAT activity to < 20% of the control level. A target sequence (CM2), mutated to be noncleavable, showed no reduction in CAT activity in the presence of the tRNA(Tyr) **ribozyme** beyond that for the antisense construct. The reduction in full-length CAT mRNA and the presence of specific cleavage products demonstrated in vivo cleavage of the target mRNA by the tRNA(Tyr) **ribozyme**. The high titer of tRNA(Tyr) **ribozyme** was a result of transcription from the RNA polymerase III promoter and led to the high **ribozyme**/substrate ratio essential for **ribozyme** efficiency.

3/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08348438 95107243 PMID: 7808396
Expression of a reporter gene is reduced by a **ribozyme** in transgenic plants.
Wegener D; Steinecke P; Herget T; Petereit I; Philipp C; Schreier P H
Max-Planck-Institut fur Zuchtungsforschung, Abteilung Genetische Grundlagen der Pflanzenzuchtung, Koln, Germany.
Molecular & general genetics : MGG (GERMANY) Nov 15 **1994**, 245 (4) p465-70, ISSN 0026-8925 Journal Code: 0125036
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

3/3,AB/20 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13459013 BIOSIS NO.: 200200087834
Expression **cassette** for antisense expression of **ribozyme**.
AUTHOR: Lieber A; Strauss M
AUTHOR ADDRESS: Berlin**Germany
JOURNAL: Official Gazette of the United States Patent and Trademark Office Patents 1205 (2):p1315 Dec. 9, **1997**
ISSN: 0098-1133
DOCUMENT TYPE: Patent
RECORD TYPE: Citation
LANGUAGE: English
1997

3/3,AB/21 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11631734 BIOSIS NO.: 199800413465
An expression vector for multiple ribozymes.
BOOK TITLE: Methods in Molecular Medicine; Therapeutic applications of ribozymes
AUTHOR: Ohkawa Jun(a); Takebe Yutaka; Taira Kazunari
BOOK AUTHOR/EDITOR: Scanlon K J: Ed
AUTHOR ADDRESS: (a)National Inst. Bioscience Human Technology, Agency Industiral Science and Technology, MITI, Tsuk**Japan
JOURNAL: Methods in Molecular Medicine 11p83-95 **1998**
BOOK PUBLISHER: Humana Press Inc., Suite 808, 999 Riverview Drive, Totowa, New Jersey 07512, USA
ISBN: 0-89603-477-1
DOCUMENT TYPE: Book
RECORD TYPE: Citation
LANGUAGE: English
1998

3/3,AB/22 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11546684 BIOSIS NO.: 199800328016
Catalytic antisense RNA based on hammerhead ribozymes.
BOOK TITLE: Practical Approach Series; Antisense technology
AUTHOR: Tabler Martin(a); Sczakiel Georg
BOOK AUTHOR/EDITOR: Lichtenstein C; Nellen W: Eds
AUTHOR ADDRESS: (a)Found. Res. Technol.-Hellas, Inst. Mol. Biol. Biotechnol., PO Box 1527, GR-711 10 Heraklion, Cre**Greece
JOURNAL: Practical Approach Series 185p93-126 **1997**
BOOK PUBLISHER: Oxford University Press, Walton Street, Oxford OX2 6DP, England
Oxford University Press, Inc., 198 Madison Avenue, New York, New York 10016, USA
ISSN: 0957-025X ISBN: 0-19-963584-6 (cloth); 0-19-963583-8 (paper)
DOCUMENT TYPE: Book
RECORD TYPE: Citation
LANGUAGE: English
1997

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? ds

Set     Items   Description
S1         97   (CCDA OR KIS OR PEMI OR PARD OR PHD OR HIGA OR CHPAI OR CH-
              PBI OR KICA OR SOC OR SOS OR SRNC OR FLMB OR PNDB OR SOF OR K-
              ORA OR KORB OR KORC OR KORD OR KORE OR KORF) AND (ANTISENSE OR
               RIBOZYM?)
S2         46   S1 AND PY<1999
S3         29   RD (unique items)
?  s antidot? and (antisens? or ribozym?) and py<1999
           32084  ANTIDOT?
           36929  ANTISENS?
            6386  RIBOZYM?
        21931987  PY<1999
      S4       7  ANTIDOT? AND (ANTISENS? OR RIBOZYM?) AND PY<1999
? rd
...completed examining records
      S5       6  RD (unique items)
? t s5/3,ab/all

 5/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

08543342   95302484   PMID: 7783193
  Mechanism of post-segregational killing by hok-homologue pnd of plasmid
R483: two translational control elements in the pnd mRNA.
  Nielsen A K; Gerdes K
  Department of Molecular Biology, Odense University, Denmark.
  Journal of molecular biology (ENGLAND) Jun 2 1995, 249 (2)
 p270-82, ISSN 0022-2836 Journal Code: 2985088R
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  The pnd system of plasmid R483 mediates plasmid stabilization by killing
of plasmid-free cells. The pnd mRNA is very stable and can be translated
into PndA protein, a cell toxin which kills the cells from within by
damaging the cell membrane. Translation of the pnd mRNA is inhibited by the
PndB antisense , a small labile RNA of 63 nt. The rapid decay of the
PndB antidote leads to onset of PndA synthesis in plasmid-free
segregants or after addition of rifampicin. Surprisingly however, the
full-length pnd mRNA was found to be translationally inactive whereas a
3'-end truncated version of it was found to be active. We have therefore
suggested previously, that the 3'-end of the full-length pnd mRNA encodes a
fold-back inhibitory sequence (fbi), which prevents its translation. Here
we present an analysis of the metabolism of the pnd mRNAs. A mutational
analysis shows that single point mutations in the fbi motif results in more
rapid truncation. The fbi mutations could not be complemented by
second-site mutations in either of the pndA or pndC Shine-Dalgarno (SD)
elements. Surprisingly, mutations in the pndC SD element also lead to a
more rapid truncation. The effect of these latter mutations was, however,
complemented by mutations in a proposed anti-SD element upstream of the
pndC SD. Mutations in the anti-SD element were lethal. These results show,
that the pnd mRNA contains two negative control elements, one located in
its very 3'-end (fbi), and one located just upstream of the pndC SD region
(the anti-SD element). These observations add to the complexity of the
induction scheme previously proposed to explain activation of pndA
expression in plasmid-free cells: In addition to its negative effect of
translation, the fbi structure also maintains a reduced processing rate in
the 3'-end of the mRNA. This permits the accumulation of a reservoir of pnd
mRNA, which can be activated by 3'-end processing in plasmid-free cells.
The anti-SD may prevent translation of the pnd mRNA during transcription,
thus preventing detrimental synthesis of toxin.
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WEST

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L7: Entry 11 of 15 File: USPT Dec 9, 1997

US-PAT-NO: 5695992
DOCUMENT-IDENTIFIER: US 5695992 A

TITLE: Expression cassette for antisense expression of ribozyme

DATE-ISSUED: December 9, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lieber; Andre	Berlin			DE
Strauss; Michael	Berlin			DE

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Max Planck Gesellschaft	Munchen			DE	03

APPL-NO: 08/ 314588 [PALM]
DATE FILED: September 28, 1994

INT-CL: [06] C12 N 15/85, C12 Q 1/68

US-CL-ISSUED: 435/320.1; 435/6, 435/91.31, 435/172.3, 536/23.1, 536/23.2, 536/24.5
US-CL-CURRENT: 435/320.1; 435/6, 435/91.31, 536/23.1, 536/23.2, 536/24.5

FIELD-OF-SEARCH: 435/91.31, 435/6, 435/320.1, 435/172.1, 435/172.3, 536/23.1, 536/23.2, 536/24.5

ART-UNIT: 189

PRIMARY-EXAMINER: LeGuyader; John L.

ABSTRACT:

The invention is an expression cassette for the antisense expression of ribozyme, having a strong promotor, suitably a T7 promotor, an adenoviral va-RNA gene, a stable loop region, and an insertion site for the antisense/ribozyme sequence in the loop region.

5 Claims, 4 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 2

BRIEF SUMMARY:

1 FIELD OF THE INVENTION

2 The present invention relates to a vector for the antisense and for the ribozyme expression. The invention can be usefully employed in molecular biology, genetic engineering, and in medical applications.

3 BACKGROUND OF THE INVENTION

4 The inactivation of gene functions by reverse genetic material is the most important methods for switching off certain genes. This is of great importance for combating infectious and other diseases (including AIDS), caused by interference with gene expression. A gene function can be inactivated on various levels: by homologous recombination at the DNA level, by antisense nucleic acids or ribozymes on the RNA level, or by antibodies on the protein level. In conversion to practice, all four possibilities have advantages and disadvantages. For therapeutic applications, only the RNA inactivation by antisense molecules, or by ribozymes appears to be implementable. Both classes of compounds can be synthesized chemically or produced in conjunction with a promotor by biological expression in vitro or even in vivo. The principle of catalytic self-cleavage of RNA molecules and the cleavage in trans has become well established in the last 10 years. The hammerhead ribozymes are characterized best among the RNA molecules with ribozyme activity. Since it was shown that hammerhead structures can be integrated into heterologous RNA sequences and that ribozyme activity can thereby be transferred to these molecules, it seems clear that catalytic antisense sequences for almost any target sequence can be created, provided the target sequence contains a potential matching cleavage site.

5 The basic principle of constructing ribozymes is very simple. An interesting region of the RNA, which contains the GUC (or CUC) triplet, is selected. Two oligonucleotides strands, each with 6 to 8 nucleotides, are taken and the catalytic hammerhead sequence is inserted between them.

6 Molecules of this type were synthesized for numerous target sequences. They showed catalytic activity in vitro and in some cases also in vivo. The best results were obtained with short ribozymes and target sequences. A topical challenge for the in vivo application is the construction of ribozyme genes which permit a continuous expression of the ribozyme in a particular cell (Bertrand, E. et al., (1994) Nucleic Acids Res. 22, 293 to 300).

7 There are five possible reasons for interference with a satisfactory functioning of expressed ribozymes within the complex intracellular milieu.

8 1. The mRNA substrate exists within the cell presumably in a highly folded structure, which furthermore can also be protected by proteins bound to parts of the structure. The encountering of accessible sites within the substrate allowing for hybridization with the complementary flanking regions of the ribozyme is a question of actual probability. Computer-aided predictions of possible, thermodynamically stable secondary structures can be useful when searching for loop regions without base pairing. However, the physiological relevance of these conformation models is still uncertain.

9 2. Since the target mRNA is transported immediately out of the cell nucleus, the ribozyme must also enter the cytoplasm, preferably along the same path. It is, however, difficult to achieve a co-localization of ribozyme and its substrate.

10 3. The in vivo use of ribozymes requires the insertion of ribozyme genes in suitable expression cassettes. The transcription of these constructs can produce mRNAs, in which the central, catalytic, secondary structure of the ribozymes is displaced by other, more stable base pairings within the non-complementary flanking sequences.

11 4. 100- to 1,000-fold excess of ribozyme molecules relative to the target sequence is necessary for attaining a recordable increase in the RNA level. The production of 10.sup.5 to 10.sup.6 ribozymes per cell over a long period of time can, however, have cytotoxic effects. In general, such high expression levels are not stable. An excess of ribozymes is needed because of the inadequate stability of the ribozymes in the presence of nucleases, because of the ineffective transport to the cytoplasm and because of the less than optimum conversion factor of the cleavage reaction.

12 5. The kinetics of the cleavage reaction and the ability of the ribozymes to carry out multiple conversion reactions depends on the binding parameters and the structure of the complementary flanking regions of the ribozymes. Cellular proteins can affect the catalysis of the cleavage reaction, probably with the help of the dissociation of the ribozyme from the substrate of the cleavage reaction, which represents the preliminary step of the next cleavage reaction. Until now, it has not been possible to predict the optimum structure of the flanking regions for a ribozyme, to guarantee high specificity and high conversion. Despite many efforts to construct specific ribozyme genes, generally only partial successes have been achieved, mostly on the basis of trial and error experimentation.

13 DESCRIPTION OF THE INVENTION

14 It is an object of the present invention to construct a vector for antisense expression and ribozyme expression. The vector shall be able to result in a continuous and stable expression of a particular desired ribozyme or an antisense sequence in a cell.

15 This objective is realized with the construction of the expression cassette of the present invention, which has a strong promotor, suitably a T7 promotor, an adenoviral va-RNA gene, a stable loop region, and an insertion site for the antisense/ribozyme sequence in the loop region.

DRAWING DESCRIPTION:

DESCRIPTION OF THE DRAWINGS

The present invention is described below in detail with reference being had to the drawing, wherein:

FIG. 1 shows the vaRNA genes, and the labelled parts show (D) the promotor, (E) the stable loop-region, and (F) the insertion site AatII of the loop region within the va-RNA, and (G) the Pstl and SALI sites in the loop region wherein the ribozymes that are to be expressed are cloned (Seq. ID. No. 2).

FIG. 2 shows the result of cleavage of hGH RNA (Seq. ID. No. 3) in vitro by a ribozyme, and the labelled parks A, B and C are described below.

DETAILED DESCRIPTION:

1 Thus, FIG. 1 shows the va-RNA gone at the site AatII into which the synthetic loop region was cloned. The ribozymes that are to be expressed are cloned between the PstI and SALI sites in the loop region. The symbolic box over the AatII site represents the sequence of the loop region, which is shown in the lower, right of FIG. 1 (open for the acceptance of ribozyme).

2 DETAILED DESCRIPTION OF THE INVENTION

3 The T7 promotor is suitably used in combination with T7 polymerase. The loop region is in a restriction site in the central part of the adenoviral va-RNA gene and its size is at least 2.times.21 bases of identical sequence. A suitable base sequence of the loop region is 5'-AACCCAGGTGTGCGACGTCAG-3' (Seq. Id. No. 1).

4 The cleavage results of FIG. 2 also show

5 (A) the structure of the specific ribozyme for a 27 n.t. region about the GUC at position 988 within the exon IV of hGH RNA;

6 (B) the maps of plasmid matrices for ribozyme synthesis by in vitro transcription with pol III (HeLa extract) and T7 RNA polymerase; and

(C) an electrophoretic representation of the cleavage products.

The invention is further described by the following specific example.

EXAMPLE

The T7Rz and T7Rzneo plasmids were linearized by a Hind III treatment. GvaRz and GvaLRz were used in circular form. hGH RNA was synthesized from a linear (SstI section) of genomic hGH gene (1663 nt) by in vitro transcription with T7 RNA polymerase (with 0.2 .mu.Ci.sup.32 P of CTP/.mu.g of RNA). An equimolar mixture (100 nM) of ribozyme and substrate was incubated at 37.degree. C. in 50 mM of Tris-HCl of pH 7.5 and 10 mM of magnesium chloride for 30 minutes with prior heat denaturation (90 seconds at 95.degree. C.). After the cleavage, the RNAs were purified and separated individually on a 6% polyacrylamide gel. Full-length RNA and ribozyme cleavage products (988 nt and 675 nt) were detected. The result shows that the embedding of the catalytic hammerhead structure in a stabilizing RNA (va) leads to a stable ribozyme, capable of functioning, only after the additional incorporation of the loop region.

SEQUENCE LISTING
(1) GENERAL INFORMATION:
(iii) NUMBER OF SEQUENCES: 3
(2) INFORMATION FOR SEQ ID NO: 1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: rRNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:
AACCCAGGTGTGCGACGTCAG21
(2) INFORMATION FOR SEQ ID NO: 2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: rRNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:
GGTTACCGCCCGCGTGTCGAACCCAGGTGTGCGACGTCAG40
(2) INFORMATION FOR SEQ ID NO: 3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleoside base
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:
UCCUCAGGAGUGGUCUUCGCCAACAGCC28

CLAIMS:

We claim:

1. An antisense and ribozyme expression cassette which comprises a strong promotor, an adenoviral va-RNA gene having a central part, a stable loop region of a size of at least 2.times.21 base pairs of identical sequences, and an insertion site for the antisense/ribozyme sequence in the loop region, wherein said promotor, the adenoviral va-RNA gene, the loop region, and the insertion site are linked as shown in FIG. 1.

2. The expression cassette of claim 1, wherein said promotor is a T7 promotor.

3. The expression cassette of claim 2, wherein the T7 promotor is employed in combination with a T7 polymerase.

4. The expression cassette of claim 1, wherein said loop region is in a restriction site in the central part of the adenoviral va-RNA gene.

5. The expression cassette of claim 1, wherein said loop region contains twice the base sequence 5'-AACCCAGGTGTGCGACGTCAG-3' (Seq. Id. No. 1).

1: X14007. E. Coli aspV gene...[gi:41016] Links

```
LOCUS       ECASPV                   284 bp    DNA     linear   BCT 14-MAY-1997
DEFINITION  E. Coli aspV gene for transfer RNA-Asp(1).
ACCESSION   X14007
VERSION     X14007.1  GI:41016
KEYWORDS    aspV gene; transfer RNA; transfer RNA-Asp.
SOURCE      Escherichia coli
  ORGANISM  Escherichia coli
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
            Enterobacteriaceae; Escherichia.
REFERENCE   1  (bases 1 to 284)
  AUTHORS   Horiuchi,T., Nagasawa,T., Takano,K. and Sekiguchi,M.
  TITLE     A newly discovered tRNA(1Asp) gene (aspV) of Escherichia coli K12
  JOURNAL   Mol. Gen. Genet. 206 (2), 356-357 (1987)
  MEDLINE   87228334
COMMENT     The sequence overlaps with that reported by Sekiya et. al. in Nucl.
            Acids Res. 8:3809-3827(1980) and by Young et. al. in J. Biol. Chem.
            254:12725-12731(1979).
            Data kindly reviewed (15-Feb-1989) by Horiuchi T.
FEATURES             Location/Qualifiers
     source          1..284
                     /organism="Escherichia coli"
                     /strain="K12"
                     /db_xref="taxon:562"
     promoter        78..83
                     /note="put. -35 region"
     promoter        102..108
                     /note="put. -10 region"
     tRNA            136..212
                     /product="tRNA-Asp"
     repeat_unit     243..252
                     /note="inverted repeat A"
     repeat_unit     256..265
                     /note="inverted repeat A'"
BASE COUNT       77 a     62 c     71 g     74 t
ORIGIN
        1 taaatacctg tgaaaggcgc taaaaatagc gacttgggcg atttttgcag caaacgattc
       61 aaaagatgag aaaaaccgtt gacgaaggtc gaggcaatcc gtaatattcg cctcgttccc
      121 aacggaacac aacgcggagc ggtagttcag tcggttagaa tacctgcctg tcacgcaggg
      181 ggtcgcgggt tcgagtcccg tccgttccgc cactattcac tcatgaaaat gagttcagag
      241 agccgcaaga tttttaattt tgcggttttt ttgtatttga attc
//
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Revised: July 5, 2002.

1: X66089. S.warneri tRNA-As...[gi:48006] Links

```
LOCUS       SWTASP                   125 bp    DNA     linear   BCT 21-JAN-1993
DEFINITION  S.warneri tRNA-Asp gene.
ACCESSION   X66089 S42075
VERSION     X66089.1  GI:48006
KEYWORDS    transfer RNA; transfer RNA-Asp; transfer RNA-Met; transfer RNA-Phe.
SOURCE      Staphylococcus warneri
  ORGANISM  Staphylococcus warneri
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 125)
  AUTHORS   McClelland,M.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-MAY-1992) M. McClelland, California Inst of Biol
            Research, 11099 North Torrey Pines Road, La Jolla CA 92037, USA
REFERENCE   2  (bases 1 to 125)
  AUTHORS   Welsh,J. and McClelland,M.
  TITLE     PCR-amplified length polymorphisms in tRNA intergenic spacers for
            categorizing staphylococci
  JOURNAL   Mol. Microbiol. 6 (12), 1673-1680 (1992)
  MEDLINE   92356831
   PUBMED   1379668
COMMENT     See X66088-90. The order of the genes is the same as in Bacillus
            subtilis and in two Mycoplasma species.
FEATURES             Location/Qualifiers
     source          1..125
                     /organism="Staphylococcus warneri"
                     /strain="CPB10E2"
                     /isolate="isolated from skin of Cercopithecus monkey"
                     /db_xref="taxon:1292"
                     /clone_lib="pBluescript SKII+"
     tRNA            1..11
                     /product="tRNA-Met"
                     /note="3' end of putative initiator tRNA-Met"
     gene            27..102
                     /gene="tRNA-Asp"
     tRNA            27..102
                     /gene="tRNA-Asp"
     tRNA            116..125
                     /product="tRNA-Phe"
                     /note="5'end of putative tRNA-Phe"
BASE COUNT       27 a     32 c     30 g     34 t      2 others
ORIGIN
        1 gcctcccgca atacatagtt ttaataggtc tcgtagtgta gcggttaaca cgcctgcctg
       61 tcacgcagga gatcgcgggt tcgaatcccg tcgagaccgc catcattatt attatggttc
      121 rrtag
//
```

Revised: July 5, 2002.

1: Z27113. H.Sapiens gene fo...[gi:415387] Links

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LOCUS       HSRNAP14K                546 bp    DNA     linear   PRI 08-JUN-1995
DEFINITION  H.Sapiens gene for RNA polymerase II subunit 14.4 kD.
ACCESSION   Z27113
VERSION     Z27113.1  GI:415387
KEYWORDS    RNA polymerase II; RNA polymerase II subunit 14.4 kD.
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 546)
  AUTHORS   Acker,J., Wintzerith,M., Vigneron,M. and Kedinger,C.
  TITLE     A 14.4 KDa acidic subunit of human RNA polymerase II with a
            putative leucine-zipper
  JOURNAL   DNA Seq. 4 (5), 329-331 (1994)
  MEDLINE   95102114
REFERENCE   2  (bases 1 to 546)
  AUTHORS   Kedinger,C.
  TITLE     Direct Submission
  JOURNAL   Submitted (09-NOV-1993) claude Kedinger, U184INSERM-LGME UPR6520
            CNRS-Inst.Chimie Biol.ULP, Strasbourg, 11, rue Humann, Strasbourg,
            Alsace, 67085, France
FEATURES             Location/Qualifiers
     source          1..546
                     /organism="Homo sapiens"
                     /db_xref="taxon:9606"
                     /cell_line="HeLa cells"
     CDS             79..462
                     /codon_start=1
                     /product="RNA Polymerase II subunit 14.4 kD"
                     /protein_id="CAA81629.1"
                     /db_xref="GI:415388"
                     /db_xref="SWISS-PROT:P41584"
                     /translation="MSDNEDNFDGDDFDDVEEDEGLDDLENAEEEGQENVEILPSGER
                     PQANQKRITTPYMTKYERARVLGTRALQIAMCAPVMVELEGETDPLLIAMKELKARKI
                     PIIIRRYLPDGSYEDWGVDELIITD"
BASE COUNT      127 a    139 c    168 g    112 t
ORIGIN
        1 gcgcgagtcg tagtgtcgct gtttgcgggt ctccgcggcg ggaccggggc gcagcggggt
       61 cgctgaggcg agggtgtcat gtcagacaac gaggacaatt ttgatggcga cgactttgat
      121 gatgtggagg aggatgaagg gctagatgac ttggagaatg ccgaagagga aggccaggag
      181 aatgtcgaga tcctcccctc tggggagcga ccgcaggcca accagaagcg aatcaccaca
      241 ccatacatga ccaagtacga gcgagcccgc gtgctgggca cccgagcgct ccagattgcg
      301 atgtgtgccc ctgtgatggt ggagctggag ggggagacag atcctctgct cattgccatg
      361 aaggaactca aggcccgaaa gatccccatc atcattcgcc gttacctgcc agatgggagc
      421 tatgaagact ggggggtgga cgagctcatc atcaccgact gagctggagt catcttcctg
      481 cccttgcccc atgcccaatt ttcattctca ctttatatgt gtaaataata aaatattcaa
      541 ctttcc
//
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Revised: July 5, 2002.

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Search Results - Record(s) 1 through 10 of 20 returned.

1. Document ID: US 6495740 B1

L4: Entry 1 of 20 File: USPT Dec 17, 2002

US-PAT-NO: 6495740
DOCUMENT-IDENTIFIER: US 6495740 B1

TITLE: Manipulation of cellulose and/or .beta.-1,4-Glucan

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

2. Document ID: US 6451603 B1

L4: Entry 2 of 20 File: USPT Sep 17, 2002

US-PAT-NO: 6451603
DOCUMENT-IDENTIFIER: US 6451603 B1

TITLE: Ribozyme nucleic acids and methods of use thereof for controlling viral pathogens

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

3. Document ID: US 6329572 B1

L4: Entry 3 of 20 File: USPT Dec 11, 2001

US-PAT-NO: 6329572
DOCUMENT-IDENTIFIER: US 6329572 B1

TITLE: Plant promoter activated by fungal infection

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

4. Document ID: US 6329518 B1

L4: Entry 4 of 20 File: USPT Dec 11, 2001

US-PAT-NO: 6329518
DOCUMENT-IDENTIFIER: US 6329518 B1

TITLE: Plant fatty acid epoxygenase genes and uses therefor

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 5. Document ID: US 6271440 B1

L4: Entry 5 of 20 File: USPT Aug 7, 2001

US-PAT-NO: 6271440
DOCUMENT-IDENTIFIER: US 6271440 B1

TITLE: Plant regulatory proteins III

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 6. Document ID: US 6215045 B1

L4: Entry 6 of 20 File: USPT Apr 10, 2001

US-PAT-NO: 6215045
DOCUMENT-IDENTIFIER: US 6215045 B1

TITLE: Developmental regulation in anther tissue of plants

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 7. Document ID: US 6177075 B1

L4: Entry 7 of 20 File: USPT Jan 23, 2001

US-PAT-NO: 6177075
DOCUMENT-IDENTIFIER: US 6177075 B1

TITLE: Insect viruses and their uses in protecting plants

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 8. Document ID: US 6127114 A

L4: Entry 8 of 20 File: USPT Oct 3, 2000

US-PAT-NO: 6127114
DOCUMENT-IDENTIFIER: US 6127114 A

TITLE: Ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Image | KWIC | Draw Desc

9. Document ID: US 6093554 A

L4: Entry 9 of 20 File: USPT Jul 25, 2000

US-PAT-NO: 6093554
DOCUMENT-IDENTIFIER: US 6093554 A

TITLE: Genetics manipulations with recombinant DNA virus comprising sequences derived from RNA virus

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Image | KWIC | Draw Desc

10. Document ID: US 5900406 A

L4: Entry 10 of 20 File: USPT May 4, 1999

US-PAT-NO: 5900406
DOCUMENT-IDENTIFIER: US 5900406 A

TITLE: Use of antibiotics of the type 2-deoxystreptamine substituted with aminosugars to inhibit growth of microorganisms containing group I introns

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Image | KWIC | Draw Desc

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@PRAY.USPT.	1109223
(3 AND (@PRAY < "1999")).USPT.	20
(@PRAY < 1999 AND (L3)).USPT.	20

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L4: Entry 8 of 20 File: USPT Oct 3, 2000

DOCUMENT-IDENTIFIER: US 6127114 A
TITLE: Ribozymes

Priority Application Year (1):
1987

Priority Application Year (2):
1988

Priority Application Year (3):
1988

Priority Application Year (4):
1988

Priority Application Year (5):
1988

Priority Application Year (6):
1988

Other Reference Publication (50):
Walbot, V. et al., (1988) "Plant Development and Ribozymes for Pathogens," Nature 334: 196-197.

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Search Results - Record(s) 11 through 20 of 20 returned.

11. Document ID: US 5856463 A

L4: Entry 11 of 20 File: USPT Jan 5, 1999

US-PAT-NO: 5856463
DOCUMENT-IDENTIFIER: US 5856463 A

TITLE: PSKH-1 ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

12. Document ID: US 5840874 A

L4: Entry 12 of 20 File: USPT Nov 24, 1998

US-PAT-NO: 5840874
DOCUMENT-IDENTIFIER: US 5840874 A

TITLE: Hammerhand ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

13. Document ID: US 5834265 A

L4: Entry 13 of 20 File: USPT Nov 10, 1998

US-PAT-NO: 5834265
DOCUMENT-IDENTIFIER: US 5834265 A

TITLE: Multifunctional RNA having self-processing activity, the preparation thereof and the use thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

14. Document ID: US 5766942 A

L4: Entry 14 of 20 File: USPT Jun 16, 1998

US-PAT-NO: 5766942
DOCUMENT-IDENTIFIER: US 5766942 A

TITLE: Ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc
Image

☐ 15. Document ID: US 5747335 A

L4: Entry 15 of 20 File: USPT May 5, 1998

US-PAT-NO: 5747335
DOCUMENT-IDENTIFIER: US 5747335 A

TITLE: Ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc
Image

☐ 16. Document ID: US 5707835 A

L4: Entry 16 of 20 File: USPT Jan 13, 1998

US-PAT-NO: 5707835
DOCUMENT-IDENTIFIER: US 5707835 A

TITLE: Ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc
Image

☐ 17. Document ID: US 5646261 A

L4: Entry 17 of 20 File: USPT Jul 8, 1997

US-PAT-NO: 5646261
DOCUMENT-IDENTIFIER: US 5646261 A

TITLE: 3'-derivatized oligonucleotide analogs with non-nucleotidic groupings, their preparation and use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc
Image

☐ 18. Document ID: US 5589580 A

L4: Entry 18 of 20 File: USPT Dec 31, 1996

US-PAT-NO: 5589580
DOCUMENT-IDENTIFIER: US 5589580 A

TITLE: Ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

19. Document ID: US 5574143 A

L4: Entry 19 of 20 File: USPT Nov 12, 1996

US-PAT-NO: 5574143
DOCUMENT-IDENTIFIER: US 5574143 A

TITLE: Ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

20. Document ID: US 5543508 A

L4: Entry 20 of 20 File: USPT Aug 6, 1996

US-PAT-NO: 5543508
DOCUMENT-IDENTIFIER: US 5543508 A

TITLE: Ribozymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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@PRAY.USPT.	1109223
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(@PRAY < 1999 AND (L3)).USPT.	20

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Search Results - Record(s) 1 through 1 of 1 returned.

☐ 1. Document ID: US 6485965 B1

L5: Entry 1 of 1 File: USPT Nov 26, 2002

US-PAT-NO: 6485965
DOCUMENT-IDENTIFIER: US 6485965 B1

TITLE: Replicating or semi-replicating viral constructs, preparation and uses for gene delivery

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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Term	Documents
"1999"[USPT]	405134
1999S[USPT]	1
RIBOZYME.USPT.	3757
RIBOZYMES.USPT.	3634
CASSETTE.USPT.	54594
CASSETTES.USPT.	20732
(RIBOZYME AND ("1999"[PRAY]) AND CASSETTE).USPT.	1
((1999)[PRAY] AND (RIBOZYME AND CASSETTE)).USPT.	1

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